Workshop: CELLULAR CHOLESTEROL HOMEOSTASIS

FIRST DEMONSTRATION BY IMMUNO-ELECTRON MICROSCOPY THAT BOTH HEPATIC AND INTESTINAL LIPOPROTEINS CONTRIBUTE TO HUMAN ATHEROSCLEROSIS

Jesus Gustavo Vazquez Figueroa[1], S. Rinehart[1], A. Mc Cree[1], H. Yi[2], P. Battey[1], T. Teramoto[1], T. Matsushima[1], M. Kinoshita[1], I. Marvasty[1], S. Voros[1]

[1]Cardiovascular Imaging, Piedmont Heart Institute, [2]Emory Univesity, Atlanta, GA, USA

Introduction: We previously showed both hepatic apoB100- and intestinal apoB48-containing lipoprotein particles in human atherosclerotic plaques by immunohistochemistry, here we evaluated plaques by immuno-electron-microscopy (IEM).

Methods: Transmission electron microscopy was performed on human liver, intestine and carotid plaque after immuno-gold labeling; tissue was fixed overnight with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and embedded in LR-white-resin. Primary antibodies were incubated overnight diluted 1:100 in PBS-containing 0.1% acetylated BSA; secondary antibodies conjugated to immuno-gold-particles were incubated for 2 hrs. Primary antibodies for apoB (Abcam 20737) and apoB100 (Abcam 50069) were commercial, polyclonal rabbit antibodies recognizing the N-terminal and C-terminal of apoB, respectively. For apoB48, we used non-commercial, murine monoclonal antibody (4C8) recognizing the C-terminal of apoB48; this region is conformationally hidden in apoB100, preventing cross-reaction.

Results: IEM confirmed the presence of ApoB in the rough endoplasmic reticulum (RER) of the hepatocytes and in the brush border of the enterocytes. ApoB100-particles were located in the RER of the hepatocytes and in the cytoplasm of the enterocytes and the Goblet cells. Interestingly, apoB48-particles were localized in the glycogen storage vesicles of the hepatocyte and in the RER of the enterocytes. ApoB-, ApoB100- and ApoB48-particles were located throughout the atherosclerotic plaque.

Conclusions: This is the first demonstration by IEM that the presence of both hepatic apoB100 and intestinal apoB48 contribute to human atherosclerosis. This may serve as clinical rationale to target both the hepatic and intestinal pathways.

Workshop: CELLULAR CHOLESTEROL HOMEOSTASIS

A NOVEL HDL-ASSOCIATED PROTEIN, PROGRANULIN, MAY PLAY AN ATHEROPROTECTIVE ROLE BY ENHANCING REVERSE CHOLESTEROL TRANSPORT

Tohru Ohama[1], R. Kawase[1], A. Matsuyama[2], H. Okura[2], T. Matsuwaki[3], M. Yuasa-Kawase[1], K. Nakatani[1], M. Inagaki[1], J. Sandoval[1], K. Tsubakio-Yamamoto[1], D. Masuda[1], Y. Nakagawa-Toyama[4], M. Nishida[4], M. Ishigami[5], Y. Ohmoto[6], N. Masugi[3], I. Komuro[1], S. Yamashita[1]

[1]Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, [2]Foundation for Biomedical Research and Innovation, Kobe, [3]Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, Tokyo, [4]Health Care Center, [5]Division of Health Science, Osaka University Graduate School of Medicine, Osaka, [6]Otsuka Pharmaceutical Co.Ltd, Tokushima, Japan

Progranulin (PGRN) is known to be involved in tumorigenesis, systemic inflammation and wound healing. However, there are few reports regarding the association of PGRN to lipid metabolism and atherosclerosis. Recently, we have found that PGRN is expressed in human monocyte-derived macrophages (Mf), secreted and exclusively bound to apolipoprotein A-I. The aim of the current study is to investigate the effect of PGRN on HDL function, especially reverse cholesterol transport (RCT). Immunohistochemical analysis indicated that PGRN is abundantly expressed in human aortic atherosclerotic plaques and is co-localized with macrophages. To analyze the effect of PGRN on the mRNA expressions of genes related to reverse cholesterol transport, we performed real time PCR after adding recombinant PGRN to human monocyte-derived macrophages (Mf). While PGRN increased the mRNA expression of ABCG1 in Mf, it did not affect that of ABCA1. We analyzed the effect of PGRN on RCT in the PGRN-KO mice. In peritoneal Mf of PGRN-KO mice, the mRNA level of ABCA1 was significantly decreased and that of ABCG1 had a tendency to decrease compared with those of wild type mice (WT-mice). In contrast, scavenger receptor class B, type I (SR-BI) and CD36 in Mf of PGRN-KO mice were significantly increased compared with those of WT-mice. These results suggest that PGRN might prevent Mf from forming foamed cells. Compared with HDL derived from plasma of WT mice, HDL from plasma of PGRN-KO mice took significantly less cholesterol from peritoneal Mf. Taken together, PGRN may play an atheroprotective role by enhancing RCT.

Workshop: CELLULAR CHOLESTEROL HOMEOSTASIS

MITOCHONDRIAL CHOLESTEROL TRAFFICKING PROTEIN, 18KDA MITOTSPO, REGULATES CHOLESTEROL EFFLUX IN HUMAN MACROPHAGES

Janice Taylor, A. Graham, Vascular Biology Group

Biological and Biomedical Science, Glasgow Caledonian University, Glasgow, UK

Removal (efflux) of excess cholesterol from macrophage 'foam cells' is central to atheroma regression and stabilisation. Increased cholesterol trafficking, from outer to inner mitochondrial membranes can enhance production of oxysterol Liver X receptor ligands by sterol 27-hydroxylase, and increase expression of genes encoding cholesterol efflux proteins, including ATP binding cassette transporters (ABCA1/G1/G4) and apoE. Mitochondrial 18kDa translocator protein (18kDa mitoTSPO)is a transmembrane cholesterol transporting protein which complexes with some constituents of the permeability transition pore (PTP). Here, we manipulate mitoTSPO gene expression, via over-expression or silencing (SiRNA), and use mitoTSPO ligands, to establish this protein as a therapeutic target capable of resolving lipid accumulation within macrophages.Over-expression of mitoTSPO ($p< 0.01$), in human (THP-1) macrophages, significantly increased gene expression of proteins implicated in mitochondrial cholesterol transport, including steroidogenic acute regulatory protein ($p< 0.05$), voltage dependent anion channel ($p< 0.05$) and adenine nucleotide transporter ($p< 0.05$). Downstream, increases in expression ($p< 0.05$) of ABCA1, ABCG1, ABCG4 and APOE genes were reflected in increased [^{3}H]cholesterol efflux to apoAI and HDL ($p< 0.01$). Macrophage cholesterol mass was significantly decreased (40%; $p< 0.05$) in cells over-expressing mitoTSPO, compared with controls. By contrast, SiRNA reduced mitoTSPO mRNA levels by 52% and inhibited cholesterol efflux. Importantly, treatment with mitoTSPO agonists PK11195 and FGIN-27 significantly increased cholesterol efflux to both apoAI and HDL (1.3 to 1.8-fold; $p< 0.05$).Thus, pharmacological targeting of the mitochondrial cholesterol trafficking machinery, exemplified by this in vitro study of mitoTSPO, may provide novel therapeutic strategies capable of resolving atheroma formation in vivo.

Workshop: CELLULAR CHOLESTEROL HOMEOSTASIS

ADIPOSE TRIGLYCERIDE LIPASE DEFICIENCY AFFECTS MACROPHAGE MORPHOLOGY AND FUNCTION AND SIGNIFICANTLY REDUCES ATHEROSCLEROSIS IN LDLR $^{-/-}$ MICE

Prakash Chandak, B. Lammers, B. Radovic, E. Aflaki, M. Buchebner, G. Haemmerle, R. Zechner, M. van Eck, D. Kratky

Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria

Recently, in adipose and non-adipose tissues adipose triglyceride lipase (ATGL) was shown to be the rate-limiting enzyme for the hydrolysis of TG. Since we found high expression of ATGL in murine macrophages and foam cells, we aimed to elucidate the impact of ATGL deficiency on macrophages morphology, function, and atherosclerosis susceptibility.

ATGL-deficient ($ATGL^{-/-}$) macrophages had significantly reduced TG hydrolase activity resulting in increased TG concentrations. ATP concentration and phagocytosis ability were markedly decreased in $ATGL^{-/-}$ macrophages compared to WT macrophages. To elucidate the effect of TG accumulation in macrophages *in vivo*, we performed bone marrow transplantation of $ATGL^{-/-}$ bone marrow into $LDLR^{-/-}$ mice. Mice were fed chow diet for 9 weeks and western type diet for another 9 weeks. Plasma TG and cholesterol levels were unaltered in $LDLR^{-/-}$ mice reconstituted with $ATGL^{-/-}$ bone marrow compared to $LDLR^{-/-}$ mice reconstituted with WT bone marrow. Notably macrophage-specific loss of ATGL resulted in a significant 43% reduction in the lesion area of $LDLR^{-/-}$ mice. TUNEL staining revealed increased apoptosis in aortic valves sections of these mice. We conclude that the absence of ATGL in macrophages is anti-atherosclerotic.

Workshop: CELLULAR IMMUNRESPONSE IN THE ARTERIAL WALL

DEFICIENCY OF THE LONG PENTRAXIN PTX3 PROMOTES VASCULAR INFLAMMATION AND ATHEROSCLEROSIS

Giuseppe Norata[1], C. Garlanda[2], A. Mantovani[2], A. Catapano[1]

[1]Department of Pharmacological Sciences, University of Milan, Milan, [2]Istituto Clinico Humanitas, IRCCS, Rozzano, Italy

Background: Immune responses participate in several phases of atherosclerosis; there is, in fact, increasing evidence that both adaptive and innate immunity tightly regulate atherogenesis. Pentraxins are a superfamily of acute phase proteins which include short pentraxins such as CRP or long pentraxins such as PTX3, a molecule acting as the humoral arm of innate immunity. To address the potential role of PTX 3 in atherogenesis, we first investigated the expression of PTX3 during atherogenesis and then we generated double knock out mice lacking PTX3 and Apo E and studied the effect of murine PTX3 deficiency on lipid metabolism, atherosclerosis development and gene expression pattern in the vascular wall.

Methods and results: PTX3 expression increases in the vascular wall of ApoE KO mice from 3 months of age up to 18 months. Double knock-out mice lacking PTX3 and Apo E were fed an atherogenic diet for 16 weeks. Aortic lesions were significantly increased in double KO and mice heterozygous for PTX3 compared to Apo E KO mice. Mice lacking PTX3 showed a more pronounced inflammatory profile in the vascular wall as detected by cDNA microarray and Q-PCR analysis and an increased macrophage accumulation within the plaque. Finally lesion size correlated with the number of bone marrow monocytes.

Conclusion: PTX3 plays atheroprotective effects in mice, which, in light of the cardioprotective effects recently reported, suggests a cardiovascular protective function of the long pentraxin 3 through the modulation of the immunoinflammatory balance in the cardiovascular system.

Workshop: CELLULAR IMMUNRESPONSE IN THE ARTERIAL WALL

SUBSTANCE P MEDIATED ADVENTITIAL MAST CELL ACTIVATION INDUCES INTRAPLAQUE HEMORRHAGE IN ADVANCED ATHEROSCLEROSIS

Ilze Bot[1], S.C.A. de Jager[1], M. Bot[1], S.H. van Heiningen[1], T.J.C. van Berkel[1], J.H. von der Thüsen[2], E.A.L. Biessen[3]

[1]Biopharmaceutics, LACDR/Leiden University, Leiden, [2]Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, [3]Pathology, University of Maastricht, Maastricht, The Netherlands

Previously, we have shown that mast cells play an important role in atherosclerotic plaque destabilization. However, the endogenous trigger for activation of these (peri)vascular cells during atherosclerosis is still unresolved.

Perivascular mast cell content (CD117 staining) correlated with the number of neurofilament^{+} nerve fibers in the adventitia of human coronary atherosclerotic plaque specimens ($P< 0.05$, $r=0.42$). Our attention turned to the neuropeptide substance P (SP) as mediator of mast cell activation. Local perivascular administration of SP (0.5 ug in pluronic gel) to advanced carotid artery plaques in apoE$^{-/-}$ mice significantly enhanced the number of adventitial mast cells compared to PBS controls (4.9 ± 0.8 versus 2.2 ± 0.5 mast cells/mm^2 tissue, $P< 0.01$). Also, mast cell activation status was increased (56 ± 9% versus 29 ± 9% in controls, $P< 0.05$). This was accompanied by a significant increase in the incidence of intraplaque hemorrhages (IPHs) in SP treated mice (5/12 versus 0/15 in controls, $P=0.01$). Co-administration of the neurokinin-1 receptor antagonist Spantide-I inhibited SP-induced mast cell activation (2.6 ± 0.4 mast cells/mm^2 tissue, $P< 0.01$), while in these mice hardly any IPHs occurred (1/16, $P=0.06$). Furthermore, SP was not effective in inducing IPHs in plaques of mast cell deficient apoE$^{-/-}$Kit(W^{-sh}/W^{-sh}) mice (1/18, $P< 0.05$), establishing the critical involvement of mast cells in SP elicited plaque destabilization.

In conclusion, our data suggest that neuropeptides such as SP can promote mast cell dependent plaque destabilization and our study provides a new, direct link between neural factors and vascular inflammation involving mast cells.

Workshop: CELLULAR IMMUNRESPONSE IN THE ARTERIAL WALL

DEFICIENT CD40-TRAF6 SIGNALING IN LEUKOCYTES PREVENTS ATHEROSCLEROSIS BY SKEWING THE IMMUNE RESPONSE TOWARDS AN ANTI-INFLAMMATORY PROFILE

Dirk lievens[1,2], L. Beckers[2], E. Wijnands[2], O. Soehnlein[1], A. Zernecke[1], T. Seijkens[2], D. Engel[2], S. Naik[3], L. Boon[4], A. Oufella[5], Z. Mallat[5], R. Noelle[6], M. de Winther[2], M. Daemen[2], E. Biessen[2], C. Weber[1], E. Lutgens[1,2]

[1]Institute for Molecular Cardiovascular Research (IMCAR), Aachen University, Aachen, Germany, [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, [3]Division of Immunology, The Netherlands Cancer Institute, Amsterdam, [4]Bioceros, Utrecht, The Netherlands, [5]Paris-Cardiovascular Research Center, Paris University, Paris, France, [6]Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA

The CD40-CD40 ligand (CD40L) signaling axis plays an important role in immunological pathways. Consequently, this dyad is involved in a plethora of chronic inflammatory diseases, including atherosclerosis. Inhibition of CD40L in apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice not only reduced atherosclerosis, but also conferred a clinically favorable plaque phenotype that was low in inflammation and high in fibrosis.

Unfortunately, blockade of CD40L may not be therapeutically feasible, as long-term inhibition will compromise systemic immune responses, or cause thromboembolic complications. Conceivably, more targeted intervention strategies in CD40 signaling will have less deleterious side effects.

Here we report that deficiency in haematopoietic CD40 reduces atherosclerosis and induces features of plaque stability. Absence of CD40-tumor necrosis factor-receptor associated factor-6 (TRAF6) but not CD40-TRAF2/3/5 signaling in MHCII^{+} cells of *Apoe*$^{-/-}$ mice abolishes atherosclerosis and confers plaque fibrosis, indicating that CD40-TRAF6 but not CD40-TRAF2/3/5 mediates the pro-atherogenic pathway. Mice with defective CD40-TRAF6 signaling display a reduced blood count of Ly6C^{high} monocytes, an impaired recruitment of Ly6C^{+} monocytes to the arterial wall, and polarization of macrophages towards an anti-inflammatory 'regulatory' M2 signature. These data unveil a role for CD40-TRAF6 but not CD40-TRAF2/3/5 interactions in atherosclerosis and establish that targeting specific components of the CD40-CD40L pathway harbors the potential to achieve therapeutic effects in atherosclerosis.

Workshop: CELLULAR IMMUNRESPONSE IN THE ARTERIAL WALL

CD137 DEFICIENCY AMELIORATE ATHEROSCLEROSIS IN THE APOLIPOPROTEIN E NULL MICE

I.-H. Jung, M.-N. Lee, J.-G. Park, J.-Y. Yoo, S.-J. Jeong, S. Jeon, M.R. Cho, E.J. Lee, **Goo Taeg Oh**

Ewha Womans University, Seoul, Republic of Korea

Background: The TNF receptor superfamily (TNFRSF), including CD40, LIGHT, and OX40, play important roles in atherosclerosis. CD137 (4-1BB), a member of the TNFRSF family, has been reported to be expressed in human atherosclerotic lesions. However, limited information is available on the precise role of CD137 in atherosclerosis and the effects of blocking CD137/CD137 ligand (CD137L) signaling on lesion formation.

Methods and results: We demonstrate that deficiency of CD137 induces a reduction in atherosclerotic plaque lesions in both LDL receptor-deficient (*Ldlr*$^{-/-}$) and Apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice which are attributed by the downregulation of cytokines such as Interferon-g (IFN-g), monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-a). CD137 signaling promotes the production of inflammatory molecules including MCP-1, Interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in endothelial cells. Stimulation of CD137L signaling activates monocytes/macrophages and augments the production of proinflammatory cytokines in atherosclerotic vessels.

Conclusions: CD137/CD137L signaling plays multiple roles in immune and endothelial cells that contribute to the progression of atherosclerosis, and thus blockade of this pathway is a promising therapeutic target for the disease.

Workshop: TREATMENT OF DYSLIPIDEMIA AND ATHEROSCLEROSIS

LIPID GOAL ATTAINMENT IN EUROPEAN ADULTS WITH DYSLIPIDAEMIA: AN ANALYSIS OF THE LIPID TREATMENT ASSESSMENT PROJECT (L-TAP) 2

Carlos Brotons[1], J. Ferrières[2], J.W. Jukema[3], D.D. Waters[4], M. Messig[5], L. Tarasenko[5], on behalf of the L-TAP 2 Investigators

[1]Sardenya Primary Health Care Center, Barcelona, Spain, [2]Rangueil Hospital, Toulouse, France, [3]Leiden University Medical Center, Leiden, The Netherlands, [4]San Francisco General Hospital and University of California, San Francisco, CA, [5]Pfizer Inc., New York, NY, USA

Objective: To study lipid goal attainment in participating European countries in the L-TAP 2 survey.

Methods: Dyslipidaemic patients aged ≥20 years on stable lipid lowering therapy in Spain, France, and the Netherlands provided blood samples at enrolment between September 2006 and April 2007. LDL-C success and HDL-C failure rates were summarized by two patient risk groups: firstly, established cardiovascular disease/diabetes mellitus (CVD/DM) and secondly primary prevention (no CVD/DM), within which SCORE evaluation (‹5% vs ≥5%) was used for further stratification. Goal attainment was benchmarked to current ESC lipid goals. Stepwise logistic regression was used for multivariate analysis.

Results: CVD/DM patients (n=1518) achieved 56% success for LDL-C ‹2.5 mmol/L and 29% for LDL-C ‹2.0 mmol/L. Primary prevention patients (n=1391) achieved 44% success for LDL-C ‹3.0 mmol/L. LDL-C success rates in primary prevention patients were similar between the ‹5% and ≥5% SCORE evaluation groups (44% vs 42%). HDL-C failure (‹1.0 mmol/L in men and ‹1.3 mmol/L in women) was higher in CVD/DM (23%) than primary prevention patients (15%). Multivariate predictors of LDL-C ‹2.5 mmol/L in CVD/DM patients were age (odds ratio [OR] 1.2 per 10-year increase) and statin therapy (OR 2.3); predictors of LDL-C ‹3.0 mmol/L in primary prevention patients were age (OR 1.2 per 10-year increase), statin therapy (OR 2.5), hypertension (OR 1.3), and SCORE ‹5% (OR 1.5).

Conclusions: This European sub-analysis of L-TAP 2 demonstrated that about half of CVD/DM patients are still not achieving ESC recommended lipid targets. These results are consistent with findings from the EuroAspire III study.

Workshop: TREATMENT OF DYSLIPIDEMIA AND ATHEROSCLEROSIS

NEW AND OLD CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS IN PATIENTS WITH CORONARY ARTERY DISEASE

Philipp Rein[1,2,3], C.H. Saely[1,2,3], A. Vonbank[1,2,3], C. Boehnel[1,3], V. Jankovic[1,3], J. Breuss[1], M. Woess[1], S. Beer[1,2,3], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Recently, an International Expert Committee concluded that haemoglobin A1c (hba1c) may be a better means of diagnosing diabetes than glucose levels. A diagnosis of diabetes was recommended with hba1c ≥6.5%. Data on the concordance of new and old criteria for the diagnosis of diabetes are very scarce; no data at all are available for patients with coronary artery disease (CAD).

Material and methods: We enrolled 1124 Caucasian patients with angiographically proven CAD who did not have previously known diabetes. An oral glucose tolerance test (oGTT) was performed in all patients.

Results: From the patients with diabetes according to the new diagnostic criterion hba1c ≥6.5% (n=110), 58 (53%) fulfilled the WHO glucose criteria for diabetes, 13 (12%) had impaired glucose tolerance (IGT), 26 (24%) impaired fasting glucose (IFG), and 13 (12%) normal fasting glucose (NFG). Conversely, the hba1c ≥6.5% criterion was fulfilled in 58 patients (63%) with diabetes according to WHO criteria, in 13 patients (11%) with IGT, in 26 patients (8%) with IFG, and in 13 patients (2%) with NFG. Compared to the standard of WHO criteria, the proposed hba1c ≥6.5% for the diagnosis of diabetes had a sensitivity of 63% and a positive predictive value of 53% for detecting previously undiagnosed diabetes, whereas specificity and negative predictive value were 95% and 97%, respectively.

Conclusions: The recently recommended hba1c criterion for the diagnosis of diabetes among CAD patients is higly specific but not sensitive. This might strongly limit its use as a screening tool for identifying individuals with diabetes.

Workshop: TREATMENT OF DYSLIPIDEMIA AND ATHEROSCLEROSIS

FAMP, A NOVEL APOA-I MIMETIC PEPTIDE PROMOTES HDL VIA ABCA1-DEPENDENT CHOLESTEROL EFFLUX

Yoshinari Uehara[1], S. Ando[2], K. Oniki[2], S. Abe[1], E. Yahiro[1], H. Tanigawa[1], S.-I. Miura[1], K. Saku[1]

[1]Department of Cardiology, [2]Department of Chemistry, Fukuoka University, Fukuoka, Japan

Apolipoprotein (apo) stimulates cholesterol efflux via the ATP-binding cassette transporter A1 (ABCA1), generating HDL and reversing the macrophage foam-cell phenotype. Despite these anti-atherogenic roles, the impact of specifically promoting ABCA1 cholesterol efflux on atherosclerosis development is not well known. Here we had developed and described the identification of FAMP, a novel apoA-I mimetic peptide which is a small peptide with 24 amino acids. In cholesterol efflux, lipid free apoA-I from human plasma and FAMP, were possible to take up cholesterol from A172 cells. Interestingly, after stimulation with T0901317 and 9cis-retinoic acid, both plasma apoA-I and FAMP mediated cholesterol efflux were drastically increased on A172 (apoA-I, 2.60-fold increase; FAMP, 3.26-fold increase, p

Workshop: TREATMENT OF DYSLIPIDEMIA AND ATHEROSCLEROSIS

PIOGLITAZONE TREATMENT REDUCES VASCULAR SENESCENCE MARKERS AND CONFERS ANTI-APOPTOTIC EFFECTS IN THE AORTIC ENDOTHELIUM IN MICE

Christian Werner, C. Gensch, J. Pöss, M. Böhm, U. Laufs

Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg, Germany

Background: PPAR-γ agonists (thiazolidinediones, TZDs) are used as insulin-sensitizing drugs for the treatment of type II diabetes mellitus. Experimental and clinical studies suggest that TZDs may mediate additional, glucose-independent vascular effects. Aging is the predominant risk factor for the development of cardiovascular disease. Telomeres and telomere-associated proteins affect aging, senescence and survival on the cellular level. Our study examines the regulation of vascular telomere biology and endothelial survival by TZDs.

Methods and results: Male C57/Bl6 mice were subjected to treatment with pioglitazone (20mg/kg*day i.p.) or vehicle for 4 weeks. Telomere repeat amplification protocol (TRAP) assays demonstrated a significant increase in aortic telomerase activity in the pioglitazone group to 319±30% vs. vehicle. Western blots showed increased protein levels of telomere-repeat binding factor 2 (367±20%) and reduced expression of the senescence- and apoptosis-related factors p16 (65±11%) and p53 (53±28%). Similar observations were made in spleen-derived mononuclear cells. Additionally, the number of endothelial progenitor cells was up-regulated in pioglitazone-treated mice (550±36%). The physiologic significance of these findings was tested by induction of vascular oxidative stress *in-vivo* by i.p. injection of lipopolysaccharide (LPS). Endothelial apoptosis in the thoracic aorta was quantified by hairpin oligonucleotid assays. LPS increased apotosis to 576±16 % of vehicle which was potently prevented in TZD-treated animals (Pio+LPS 241±12 %). All reported effects $p < 0.05$, n =6-12 per group.

Conclusions: Pioglitazone treatment up-regulates telomerase activity, telomere-stabilizing proteins and induces anti-senescent effects in the vascular wall. The reduction of LPS-induced endothelial apoptosis underscores that the observed effects are physiologically relevant in mice.

Workshop: BIOLOGY OF LIPOPROTEIN RECEPTORS

A ROLE FOR SCAVENGER RECEPTOR BI IN HUMAN BIOLOGY

M. Vergeer[1], S.J.A. Korporaal[2], R. Franssen[1], I. Meurs[2], R. Out[2], G.K. Hovingh[1], J.A. Sierts[3], G.M. Dallinga[3], A.G. Holleboom[1], T.J.C. van Berkel[2], J.J.P. Kastelein[1], M. van Eck[2], **Jan Albert Kuivenhoven**[1,3]

[1]Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, [2]Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Leiden, [3]Experimental Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands

Background: In mice, scavenger receptor class B type I (SR-BI) is a receptor of high-density lipoprotein cholesterol (HDL-C) that is involved in cellular cholesterol efflux, platelet function and adrenal steroidogenesis. Despite elevated HDL-C levels, SR-BI knockout mice are susceptible to atherosclerosis. It is presently unknown whether SR-BI is relevant for human biology.

Methods and results: To study the relevance of SR-BI in man, we sequenced the SR-BI gene in 162 subjects with elevated HDL-C levels and identified 1 person carrying a missense mutation (P297S). The functionality of this mutation was established in *in vivo* and *in vitro* experiments. We identified 19 SR-BI^{P297S} carriers in the proband's family, who were characterized by high HDL-C levels (allelic effect +0.50 mmol/L [95%CI 0.30 - 0.70], p< 0.001), and after adjustment for cardiovascular risk factors, increased carotid atherosclerosis (intima-media thickness +91 µm [95%CI 19 - 164], p=0.016). Their macrophages had a reduced capacity to efflux excess cholesterol while their platelets displayed increased cholesterol content and impaired function. Finally, adrenal steroidogenesis in carriers was attenuated, as evidenced by decreased urinary excretion of sterol metabolites, a decreased response to tetracosactin and evidence of adrenal insufficiency.

Conclusions: This report on a family with a functional mutation in SR-BI supports an important and clinically relevant role for SR-BI in human biology. The data highlight a role for HDL in human adrenal steroidogenesis and the notion that higher HDL-C levels do not necessarily protect from atherosclerosis. Both these aspects merit caution regarding the development of HDL increasing therapies.

Workshop: BIOLOGY OF LIPOPROTEIN RECEPTORS

A COMMON POLYMORPHISM IN LDL-RECEPTOR GENE IS A PREDICTOR OF FACTOR VIII ACTIVITY AND IS ASSOCIATED WITH CORONARY ARTERY DISEASE

Nicola Martinelli[1], B. Lunghi[2], D. Girelli[1], M. Pinotti[2], G. Marchetti[2], M. Bisi[2], F. Manzato[3], R. Corrocher[1], O. Olivieri[1], F. Bernardi[2]

[1]Department of Clinical and Experimental Medicine, University of Verona, Verona, [2]Department of Biochemistry and Molecular Biology, University of Ferrara, Ferrara, [3]Ospedale di Mantova, Mantova, Italy

Background: A few genetic polymorphisms have been demonstrated to contribute to factor VIII activity (FVIII:C) broad variability. Observations in mouse models suggest that low-density lipoprotein receptor (LDLR) may play a role in regulating FVIII plasma levels. In the current study we assessed the association between a common polymorphism of the LDLR gene (rs688, C to T transition in the exon 12) and FVIII:C levels in a subset of patients from the angiography-based Verona Heart Study, with or without coronary artery disease (CAD).

Methods: A total number of 983 subjects (692 CAD and 291 CAD-free) not assuming anticoagulants were included in the present study.

Results: The carriers of T allele presented higher FVIII:C levels than CC homozygotes (168± 54 *versus* 160± 51 IU/dL, *P*=0.031 by t-test). In a linear regression model adjusted for sex, age, blood group, inflammation status and renal function, the carriership of T allele remained an independent determinant of FVIII:C levels (standardized beta-coefficient 0.079, *P*=0.013). On the other hand, no significant association was found between the rs688 and plasma lipid concentrations. Consistently with FVIII:C levels, the carriership of T allele was more represented in CAD patients than in CAD-free subjects (69.9% versus 62.5%, *P*=0.023) and remained significantly associated with CAD after adjustment for all the traditional atherosclerosis risk factors, including lipid profile (OR 1.53 with 95%CI 1.04-2.25, *P*=0.031).

Conclusions: Our data suggest that the LDLR rs688 genotype, modulating splicing efficiency, predicts FVIII:C levels. Carriers of T allele may have an increased risk of CAD independently from cholesterol levels.

Workshop: BIOLOGY OF LIPOPROTEIN RECEPTORS

THE ANTIPROLIFERATIVE EFFECT OF LRP6 AND ITS IMPAIRMENT BY R611C MUTATION ARE MEDIATED BY INDEPENDENT INTERMEDIATES OF THE PDGF PATHWAY

Ali Keramati[1], R. Singh[1], W. Liu[1], S. Faramarzi[1], T. Nottoli[1], G. Tellides[2], A. Mani[1]

[1]Cardiovascular Medicine Department, [2]Cardiothoracic Surgery Department, Yale University School of Medicine, New Haven, CT, USA

We recently identified a disease causing mutation (R611C) at a highly conserved residue of the LDL receptor like protein 6 (LRP6) in a family with early onset coronary artery disease (CAD), and metabolic syndrome. This finding signifies the effect of a single gene defect in LRP6 on development of atherosclerosis. The exact mechanisms linking LRP6 mutation with atherosclerosis are not understood. PDGF signaling plays a key role in vascular smooth muscle proliferation and migration which are two major pathological components of advanced atherosclerotic lesions. Expression levels of a number of PDGF signal peptides are substantially higher in the lymphoblastoid cells from LRP6 mutation carriers compared to those of the non-carriers. We demonstrated that wildtype LRP6 forms a complex with PDGFR-B, reduces its protein level by lysosomal degradation and diminishes cellular proliferation. Through dissection of PDGF-PDGFR signaling we demonstrated that wildtype and mutant LRP6 regulate cell cycle activity by triggering distinctive PDGF dependent pathways. This effect is mainly accounted for by overexpression and activation of STAT1 and cycline D1. The GSK3 activator OHDA abolishes the proliferative effect of the mutant LRP6 by reducing cycline D1. The in vivo correlation of these findings is the markedly increased and colocalization of LRP6 and PDGFR-B in human atherosclerotic coronary arteries. These findings implicate LRP6 as a critical modulator of cell cycle activation of smooth muscle cells and a potential target for therapeutic interventions in atherosclerosis.

Workshop: BIOLOGY OF LIPOPROTEIN RECEPTORS

HIGH SPEED INTRAVITAL MICROSCOPY VISUALIZES THE UPTAKE OF NANOCRYSTALS-LABELLED TRIGLYCERIDE-RICH LIPOPROTEINS IN STELLATE CELLS *IN VIVO*

O. Bruns[1], A. Bartelt[1], M. Calvo[2], C. Enrich[2], C. Waurisch[3], A. Eychmüller[3], R. Reimer[4], H. Hohenberg[4], U. Beisiegel[1], **Joerg Heeren**[1]

[1]IBMII: Molecular Cell Biology, University Medical Center Hamburg Eppendorf, Hamburg, Germany, [2]Facultat de Medicina, Universitat de Barcelona, IDIBAPS, Barcelona, Spain, [3]Physical Chemistry and Electrochemistry, Technical University Dresden, Dresden, [4]Electron Microscopy and Microtechnology, Heinrich-Pette-Institute, Hamburg, Germany

Objective: The binding and uptake of proatherogenic triglyceride-rich remnant lipoproteins (TRL) into the liver is a complex biphasic process. It is thought that TRL are first sequestered within the Space of Disse, which is filled with heparan sulfate proteoglycanes and stellate cells, before they are internalized by hepatocytes via apolipoprotein E (apoE) -dependent lipoprotein receptor mediated endocytosis. Here we visualize the trapping of TRL with the liver in wild-type, apoE and LDL receptor deficient mice by high-speed intravital microscopy *in vivo*.

Methods: Hydrophobic, fluorescent nanocrystals were embedded into the lipid core of TRL. Human LDL and TRL were labelled with fluorescent lipids (DiI, DiD). Mice were anaesthetised, a tail vein catheter was inserted and a liver lobule was prepared via a small incision. Lipoproteins were injected and uptake was analyzed by high-speed intravital microscopy. Results were correlated with conventional confocal and electron microscopy.

Results: Cellular uptake of lipoproteins could be followed with a time resolution of 25 frames per second. Lipoproteins were detected directly after injection in the Space of Disse. In contrast to LDL, which was predominantly taken up by hepatocytes, TRL were directly internalized by stellate cells. This uptake was confirmed by electron microscopy and was independent of the LDL receptor and apoE.

Conclusion: The initial step of hepatic TRL uptake involves stellate cells as a so far underestimated cellular component in TRL clearance. To further understand the complexity of chylomicron remnant metabolism the role of stellate cell needs to be further elucidated on the molecular level.

Workshop: INFLAMMATION AND ITS CONSEQUENCES

INTERLEUKIN-6 STIMULATES ABCA1-MEDIATED CHOLESTEROL EFFLUX AND PHAGOCYTOSIS OF APOPTOTIC CELLS IN HUMAN MACROPHAGES AND PROMOTES AN ANTI-INFLAMMATORY M2 PHENOTYPE

E. Frisdal, P. Lesnik, M. Olivier, P. Robillard, M.J. Chapman, M. Guerin, **Wilfried Le Goff**

INSERM UMR S939, Paris, France

Cholesterol-laden macrophages originating from ingestion of either modified lipoproteins or apoptotic cells constitute typical phagocytes present in early and advanced atherosclerotic lesions, respectively. The ATP-binding cassette A1 transporter (ABCA1) promotes macrophage cholesterol efflux to apolipoprotein AI (apoAI), thereby attenuating foam cell formation and atherosclerotic plaque progression. Interleukin-6 (IL-6) is a macrophage secretory product which may modulate intraplaque inflammation, but whose precise role in atherogenesis is unclear. We therefore evaluated the impact of IL-6 on cholesterol homeostasis in human monocyte-macrophages and THP-1 cells. ABCA1-mediated cholesterol efflux to apoAI was significantly increased (2.5-fold) by IL-6 (50 ng/mL) subsequent to the induction of ABCA1 mRNA and protein levels. Stimulation of ABCA1-mediated cholesterol efflux by IL-6 was however abolished by specific inhibition of Jak-2 or the Jak-2/Stat3 signalling pathway. In addition, IL-6 enhanced phagocytosis of apoptotic Jurkat cells in THP-1 macrophages, an effect abolished by either a Jak-2 inhibitor or glyburide, an inhibitor of ABCA1 activity. Moreover, ABCA1-mediated efflux of cholesterol from apoptotic cell-loaded phagocytes was equally induced by IL-6. Finally, IL-6 polarized human macrophages towards a M2 phenotype characterized by an anti-inflammatory cytokine profile.

Thus, activation of human monocyte-macrophages by IL-6 may favour reduction in both foam cell formation and thus growth of the necrotic plaque core, potentially contributing to enhanced plaque stability.

Workshop: INFLAMMATION AND ITS CONSEQUENCES

ADVERSE MATRIX METALLOPROTEINASE PRODUCTION IN CLASSICALLY COMPARED TO ALTERNATIVELY ACTIVATED HUMAN MACROPHAGES

Wei-Chun Huang[1,2], G.B. Sala-Newby[1], A.C. Newby[1]

[1]Bristol Heart Institute, University of Bristol, Bristol, UK, [2]Cardiovascular Center, Kaohsiung Veteran General Hospital, Kaohsiung, Taiwan R.O.C.

Background and aims: Overproduction of matrix metalloproteinases (MMPs) from foam cell macrophages (FCMs) occurs in atherosclerotic plaques. Recently we identified functionally-distinct $MMP14^{+}TIMP3^{-}$ and $MMP14^{-}TIMP3^{+}$ FCM populations in rabbits but the underlying mechanisms and their existence in man remained uncertain.

Methods: We studied MMPs expression in macrophages differentiated for 7 days from total human monocytes or selected $CD16^{-}$ monocytes derived from donor buffy coats using quantitative RT-PCR and western blotting. All experiments were replicated 5-7 times. Macrophages were classically activated with bacterial lipopolysaccharide and interferon-γ for 18 hours or alternatively activated with interleukin-4 throughout differentiation.

Results: Differentiation to macrophages increased expression of MMPs-7, -9, -12 -14 and TIMP-3 by 4900, 1400, 79, 63 and 400-fold (all $p< 0.003$). Classical activation up-regulated expression of MMP-1, -10, -12, and -14 by 10, 12, 4 and 8-fold; and down-regulated TIMP-3 by 2-fold. Alternative activation of macrophages increased MMP-12 and TIMP-3 by 3.7 and 2.3-fold and down-regulated MMP-1, -2, -7 and -9 plus TIMP-1 expression by 5, 99, 20, 20 and 5-fold. Changes in MMP-10, -12, -14 and TIMP-3 were confirmed at the protein level. There were no consistent differences between unselected and $CD16^{-}$ monocyte-derive macrophages.

Conclusions: Macrophage differentiation from monocytes and their classical or alternative activation profoundly affects the expression profile of MMPs and TIMPs. Classical activation favours $MMP14^{+}TIMP3^{-}$ macrophages while alternative activation favours $MMP14^{-}TIMP3^{+}$ populations. The greater propensity of $MMP14^{+}TIMP3^{-}$ FCMs to destroy and invade the ECM, proliferate, and undergo apoptosis suggests that classically activated macrophages are culprits in plaque instability.

Workshop: INFLAMMATION AND ITS CONSEQUENCES

ROLE OF FCXRIIA GENOTYPE FOR THE PROINFLAMMATORY EFFECTS OF C-REACTIVE PROTEIN ON HUMAN ENDOTHELIAL CELLS

Dorette Raaz-Schrauder[1], A.B. Ekici[2], I. Cicha[1], L. Klinghammer[1], M. Herrmann[3], W.G. Daniel[1], A. Reis[2], C.D. Garlichs[1]

[1]Cardiology and Angiology, [2]Institute of Human Genetics, [3]Medical Clinic III, University Hospital Erlangen, Erlangen, Germany

Background: The biological effects of CRP are mainly transduced via FcχRIIa, a receptor well established to interact with CRP and the Fc portion of IgG. FcχRIIa exists in different genotypes bearing either an arginine (R131) or a histidine (H131) at the extracellular amino acid position 131 of the mature protein. Detailed functional analyses has shown that only FcχRIIa R131 displays high avidity for CRP. Therefore, our study investigates the role of the FcχRIIa genotype for the proinflammatory effects of CRP on endothelial cells.

Methods and results: We tested the effects of CRP on the expression of adhesion molecules in cultured human umbilical vein endothelial cells (HUVEC) depending on the FcχRIIa genotype (FcχRIIa-H/H-131, FcχRIIa-H/R, FcχRIIa-R/R). The expression of ICAM-1, VCAM-1, and E-Selectin on HUVEC was detected by flow cytometry. Recombinant human CRP showed significantly different effects on HUVEC depending on their FcχRIIa genotype: ICAM-1 expression was significantly upregulated by about 30% in HUVEC with the H/R-genotype as compared to HUVEC with H/H. In contrast, there were no significant differences of the stimulatory effects of CRP on VCAM-1 and E-Selectin expression in HUVEC with FcχRIIa-H/H-131 genotype compared to H/R-131.

Conclusions: Our data show that CRP directly influences the expression of adhesion molecules via its interaction with FcχRIIa. We found a dependency between the FcχRIIa genotype and the stimulatory capacity of CRP in HUVEC, i.e. the expression of ICAM-1 was strongly elevated in the FcχRIIa H/R-genotype. This finding broadens our understanding for the genetic susceptibility for atherosclerosis in the presence of systemic inflammation.

Workshop: INFLAMMATION AND ITS CONSEQUENCES

NFKB2-DEFICIENCY IS ASSOCIATED WITH INCREASED ATHEROSCLEROSIS IN APOE$^{-/-}$ AND LDLR$^{-/-}$ MICE

Sarah Dühring[1], L. Holdt[1], J. Ernst[1], F. Weih[2], J. Thiery[1], D. Teupser[1]

[1]Institute of Laboratory Medicine, University Leipzig, Leipzig, [2]Leibniz Institute for Age Research, Jena, Germany

Objectives: Inflammatory processes mark all stages of atherogenesis. The NFkB pathway represents one of the key regulators of inflammation. Recently, an alternative NFkB pathway with NFkB2 and RelB representing the terminal transcription factors has been described. The aim of the present study was to investigate the influence of alternative NFkB signalling on atherosclerotic lesion formation in NFkB2 knockout mice.

Methods and results: NFkB2-deficient mice on the atherosclerosis-sensitizing ApoE$^{-/-}$ and LDLR$^{-/-}$ backgrounds were fed a standardized semisynthetic diet and sacrificed at 20 and 24 weeks of age. Atherosclerotic lesion area was consistently elevated at the brachiocephalic artery (BCA) of NFkB2$^{-/-}$ mice compared to NFkB2$^{+/+}$ controls in ApoE$^{-/-}$ and LDLR$^{-/-}$ mice. Lesion area was also elevated at the aortic root, even though this difference did not reach statistical significance. Consistent with enhanced atherosclerotic lesion formation at the BCA, expression analysis of NFkB2 revealed significantly elevated NFkB2 expression in the BCA of NFkB2$^{+/+}$ mice. Microarray expression analysis (Illumina Mouse Ref-8) revealed 1124 differentially expressed genes between NFkB2$^{-/-}$ and NFkB2$^{+/+}$ bone marrow derived macrophages ($P < 0.001$). The strongest difference was seen for matrix metalloproteinase 9 (MMP9) which was 15-fold increased in NFkB2$^{-/-}$ animals compared to NFkB2$^{+/+}$ controls ($P < 10^{-7}$). Differences in MMP9 expression could be confirmed in macrophages from NFkB2$^{+/+}$, NFkB2$^{+/-}$, NFkB2$^{-/-}$ mice.

Conclusion: Atherosclerotic lesion area was consistently elevated in the BCA and aortic root of NFkB2-deficient ApoE$^{-/-}$ and LDLR$^{-/-}$ mice. This effect might at least in part be mediated through MMP9 which was significantly higher expressed in macrophages of NFkB2$^{-/-}$ mice.

Workshop: MANAGING THE SOCIOECONOMIC AND ENVIRONMENTAL RISK FACTORS

LONG-TERM RESIDENTIAL EXPOSURE TO FINE PARTICULATE MATTER AND TRAFFIC INDEPENDENTLY INCREASE ARTERIAL BLOOD PRESSURE

Kateryna Fuks[1], A. Viehmann[1], S. Möhlenkamp[2], S. Moebus[1], H. Jakobs[3], C. Kessler[3], R. Erbel[2], K.-H. Jöckel[1], B. Hoffmann[1], on behalf of the Heinz Nixdorf RECALL Investigator Group

[1]Institute for Medical Informatics, Biometry and Epidemiology, University Hospital of Essen, [2]West German Heart Centre of Essen, University Duisburg-Essen, Essen, [3]Rhenish Institute for Environmental Research, University of Cologne, Cologne, Germany

Introduction: Recent evidence suggests that long-term exposure to fine particulate matter ($PM_{2.5}$) and high traffic enhances atherogenesis. We investigate whether this effect may be mediated by chronic increases in arterial blood pressure (BP).

Methods: We used baseline data (2000-2003) on 4352 participants, aged 45-75 years, from the Heinz Nixdorf Recall Study, a population-based prospective cohort in Germany. Exposure to $PM_{2.5}$ ($\mu g/m^3$) was assessed using a dispersion and chemistry transport model. Long-term exposure to traffic was assessed as distance between residence and next major road. Arterial BP was measured with an automated oscillometric device. Linear regression and generalized additive models were used, including distance to traffic, long- (365-day moving average) and short-term (2-day moving average) $PM_{2.5}$, time trend, season, temperature, and personal characteristics.

Results: In the fully-adjusted model, we observed a traffic-independent increase in systolic and diastolic BP of 1.7 mmHg (95%CI: 0.6-2.7) and 0.9 mmHg (0.3-1.5), respectively, per interquartile increase in $PM_{2.5}$ (2.4 $\mu g/m^3$). Compared with participants living >200 m away from a major road, participants living within 50 (n=108), 51 to 100 (n=202), and 101 to 200 m (n=336) had an increase in systolic BP of 2.4 (95%CI: -1.1-6.0), 1.1 (-1.5-3.8), and 1.0 mmHg (-1.1-3.1), respectively. Stronger effects were generally found in women and elderly.

Conclusions: These results suggest that long-term exposure to urban air pollution at levels below current regulatory standards and long-term exposure to high traffic may chronically increase arterial BP. This effect can contribute to the observed association of air pollution with atherosclerosis.

Workshop: MANAGING THE SOCIOECONOMIC AND ENVIRONMENTAL RISK FACTORS

QUALITY OF LIFE OF CHILDREN WITH FAMILIAL HYPERLIPIDEMIAS AFTER QUANTITATIVE OR QUALITATIVE DIETARY RESTRICTION

Arrigo Cicero[1], B. Gerocarni[1], G. Derosa[2], C. Borghi[1]

[1]Internal Medicine, Aging and Kidney Disease Dept., University of Bologna, Bologna, [2]Internal Medicine and Therapeutics Dept., University of Pavia, Pavia, Italy

Background: The diagnosis of Familial Hyperlipidemia increase the anxiety level of children and decrease their perceived quality of life. In particular, the change in dietary habit prescribed to manage the hyperlipidemia could strongly modify their life-style.

Objective: To evaluate the impact of a quantitative and a qualitative dietary program on their metabolic parameters and anxiety level.

Methods: We enrolled 42 patients, mean age 13.4±2.9 years old firstly diagnosed for Familial Dyslipidemia in the setting of a Lipid Clinic. We investigated the factors associated to worsening in children perceived quality of life after quantitative or qualitative dietary changes. For children, mean scores on the Child Behavior Checklist (CBCL), the Children's Depression Inventory (CDI) and the State-Trait Anxiety Inventory for Children (STAIC). Mothers psychological symptoms were measured by the Hopkins Symptom Checklist.

Results: The effects of the two diets was similar in term of diet improvement cholesterolemia reduction, even if children continuing the quantitative diet loss more weight than the other ones. As it regards the children mood, adjusting data for age and sex of the child and mother's age and education level, higher CBCL scores are significantly associate to the permanence in quantitative diet, higher age of the children, higher CDI and STAIC score, and to mother anxiety at the baseline.

Conclusions: In children with Familial Hyperlipidaemia a dietary program based on quality of food choice is associated to a more healthy children behavior than a quantitative one. However this result is particularly influenced from the baseline mother anxiety level.

Workshop: MANAGING THE SOCIOECONOMIC AND ENVIRONMENTAL RISK FACTORS

PERSISTENT DEPRESSIVE SYMPTOMS ARE ASSOCIATED WITH CORONARY ARTERY CALCIFICATION

Mark Hamer[1], M. Kivimaki[1], A. Lahiri[2], A. Steptoe[1]

[1]Epidemiology and Public Health, University College London, [2]Cardiac Imaging and Research Centre, Wellington Hospital, London, UK

Introduction: Tthe assessment of sub-clinical atherosclerosis before clinical disease is manifest helps delineate the temporal relationship between depression and CHD. However, the association between depression and sub-clinical atherosclerosis remains unclear. By assessing depressive symptoms only at one point in time, most previous studies have failed to ascertain long-term exposure.

Objectives: We examined the association of long-term depressive symptoms assessed at 3 time points (over 10 yrs) with a marker of sub-clinical atherosclerosis assessed in terms of coronary artery calcification (CAC).

Methods: Participants were 454 healthy men and women (aged 53 to 76 yrs) from the Whitehall II epidemiological cohort. Measures of CAC were taken using electron beam computed tomography at the final follow up assessment and we used the 30 item General Health Questionnaire to assess cognitive symptoms of depression.

Results: Clinically relevant levels of CAC (Agatston score>100) were detected in 25.3% of the sample and 18.9% of the sample reported depressive symptoms at least once during follow up. Participants that were persistently depressed had over a two fold increased risk of significant CAC (odds ratio = 2.36, 95% CI, 1.04-5.35) compared with never depressed after adjustment for socio-demographic and conventional cardiac risk factors. In gender stratified analyses this association persisted in men only. Participants that reported only being depressed on one occasion were not at elevated risk of CAC.

Conclusions: Persistent cognitive symptoms of depression assessed over several time points, but not on a single occasion, are related to sub-clinical coronary atherosclerosis in men free of overt CHD.

Workshop: RISK DETECTION, BIOMARKERS AND IMAGING

CAROTID INTIMA-MEDIA THICKNESS IS NOT INDEPENDENTLY PREDICTIVE FOR HARD CORONARY EVENTS IN MEN AND WOMEN -THE HEINZ NIXDORF RECALL STUDY

Marcus Bauer[1], B. Hoffmann[2], S. Möhlenkamp[1], N. Lehmann[2], U. Roggenbuck[2], A. Schmermund[3], S. Moebus[2], N. Dragano[4], M. Bröcker-Preuss[5], A. Stang[6], K. Mann[7], J. Siegrist[8], K.-H. Jöckel[2], R. Erbel[1], on behalf of the Heinz Nixdorf Recall Study Investigative Group

[1]Department of Cardiology, West-German Heart Center, University Duisburg-Essen, [2]Institute for Medical Informatics, Biometry and Epidemiology, University Duisburg-Essen, Essen, [3]CCB - Cardioangiologisches Centrum Bethanien, Frankfurt/Main, [4]Department of Medical Sociology, Heinrich-Heine University Düsseldorf, Düsseldorf, [5]Department of Clinical Chemistry and Laboratory Medicine, University Hospital of Essen, Essen, [6]Institute of Clinical Epidemiology, Medical Faculty, Martin-Luther-University of Halle-Wittenberg, Halle, [7]Department of Endocrinology and Division of Laboratory Research, University of Duisburg-Essen, Essen, [8]Department of Medical Sociology, University of Düsseldorf, Düsseldorf, Germany

Background: The increase of intima-media thickness (IMT) is a marker of subclinical atherosclerosis and may improve prediction of cardiovascular events. Population-based data about the predictive value of IMT in regard to hard coronary events are rare.

Methods: IMT was assessed with B-mode ultrasonography and a 10- MHz transducer. IMT was calculated manually in 3552 subjects, aged 45-75 years, (59±8 yrs.; 51% women) without known coronary artery disease from the Heinz Nixdorf Recall Study. Subjects were followed for 5 years for the occurrence of coronary death or non-fatal myocardial infarction (n=57 in men, n=20 in women).

Results: In univariate logistic regression analysis IMT was positively associated with hard coronary events (odds ratio (OR, per 0.1mm, [95% CI]) =1.45 [1.26-1.67], p< 0.0001). Accordingly, the mean value of IMT was higher in subjects with events vs. subjects without events. This relation was stronger in men (0.77± 0.15mm (events) vs. 0.70±0.14 (non-events); p< 0.001) than in women (0.68±0.10 (events) vs. 0.64±0.11 (non-events); p=0.14). When adjusting for age and gender, the positive association attenuated (OR=1.25 [1.06-1.47], p=0.008). After adjustment for coronary risk factors (Hx. of diabetes, total cholesterol, systolic blood pressure, smoking, HDL, BMI), the association further diminished (OR=1.18 [1.00-1.40], p=0.06). Stratified by gender, we only saw a weak association in men (OR=1.19 [0.98-1.44], p=0.07), but not in women (OR=1.17 [0.79-1.74], p=0.43).

Conclusion: The predictive value of IMT for hard coronary events is largely explained by coexistence of traditional cardiovascular risk factors. The specific role of IMT in coronary risk stratification remains to be defined.

Workshop: RISK DETECTION, BIOMARKERS AND IMAGING

LIMITS IN DETECTION OF HIGH-RISK ATHEROSCLEROTIC PLAQUES WITH USPIO-ENHANCED MRI AT 3T IN AN ANIMAL MODEL OF ATHEROSCLEROSIS

Harald Ittrich[1], C. Jahntz[1], K. Peldschus[1], B. Misselwitz[2], C. Weber[1], G. Adam[1]

[1]Dept. of Diagnostic and Interventional Radiology, University Hospital Hamburg-Eppendorf, Hamburg, [2]Research Laboratories, Bayer Schering Pharma AG, Berlin, Germany

Purpose: To test the potential of 3T MRI for detection of high-risk atherosclerotic aortic plaques after injection of different USPIO dosages.

Material and methods: MRI of the aorta was performed on 15 WHHL and 6 NZW rabbits (control) before and after USPIO administration (DDM 43/34, Schering, Germany) at 3T. 3 groups with i.v. dosages of 1.0, 0.25 and 0.1 Fe/kg were used (5 WHHL/2 NZW per dose). Transverse T2*w 3D FFE and parasagittal T1w 3D MRA covering the aorta were used to investigate areas of signal intensity (SI) reduction within the aortic wall demonstrating the uptake of USPIO. Images were matched with histology (HE, Prussian blue, macrophage antibody RAM11).

Results: SI reduction ($p < 0.05$) within the aortic wall of WHHL were detected after injection of 1.0mmol/kg (4/15) correlated with USPIOs in macrophages of inflammatory plaques (double-immunostaining). No significant SI reduction was detected in WHHL after injection of 0.25 and 0.1mmol/kg (11/15) with negligible USPIO uptake despite the high burden of atherosclerotic plaques. Animals of the control group (NZW) showed no aortic SI changes (6/6) corresponding to the absence of plaques/macrophages. Increasing the detection sensitivity for USPIO increased also artificial SI drops from bone marrow of vertebrae/ribs or lymph nodes.

Conclusion: Contrast enhanced MRI techniques at 3T using experimental dosages of USPIO (1.0mmol/kg) allow identification of unstable atherosclerotic plaques due to USPIO accumulation in macrophages. The lack of aortic SI reductionafter clinically relevant dosages of USPIO ((0.25),0.1mmol/kg) does not correlate with the absence of plaque formations due to low USPIO uptake.

Workshop: RISK DETECTION, BIOMARKERS AND IMAGING

DISCORDANCE BETWEEN LDL CHOLESTEROL AND LDL PARTICLE CONCENTRATION IN RELATION TO INCIDENT CVD EVENTS IN 27673 WOMEN

Samia Mora[1], J. Otvos[2], P. Ridker[3]

[1]Preventive Medicine, Brigham and Women's Hospital, Boston, MA, [2]LipoScience, Inc, Raleigh, NC, [3]Brigham and Women's Hospital, Boston, MA, USA

Background: The cholesterol content of LDL particles varies between individuals because of differences in size and relative content of cholesterol ester and triglycerides. We aimed to determine whether discordance in levels of LDL cholesterol (LDL-C) and LDL particle concentration (LDL-P) is related to CVD.

Methods and results: Baseline LDL-C was measured directly and LDL-P measured using nuclear magnetic resonance spectroscopy (NMR) in 27673 apparently healthy middle-aged women with 11-year follow-up. Participants were categorized as concordant if LDL-C and LDL-P differed by < ±20 percentile units (N=16712; mean LDL-C 124 mg/dL, mean LDL-P 1365 nmol/L), discordant-high LDL-C if LDL-C was higher than LDL-P by ≥ 20 percentile units (N=5570; mean LDL-C 144 mg/dL, mean LDL-P 1118 nmol/L), and discordant-high LDL-P if LDL-P was higher by ≥ 20 percentile units (N=5391; mean LDL-C 105 mg/dL, mean LDL-P 1624 nmol/L). Women with discordant-high LDL-P had significantly higher (P< 0.001) BMI, metabolic syndrome, and C-reactive protein than women who were concordant or discordant-high LDL-C. Despite lower LDL-C levels, the risk of incident CVD was highest in women with discordant-high LDL-P. Conversely, despite higher LDL-C levels, CVD risk was lowest in women with discordant-high LDL-C. Using the concordant group as reference, the hazard ratio for CVD in the discordant-high LDL-P group was 1.46 (95% CI 1.26-1.68), and in the discordant-high LDL-C group 0.78 (95% CI 0.65-0.93).

Conclusion: Discordance between LDL-C and LDL-P levels was associated with CVD risk, with disproportionately higher LDL-P having higher risk, and disproportionately higher LDL-C having lower risk, than concordant levels.

Workshop: RISK DETECTION, BIOMARKERS AND IMAGING

LIPID LOWERING THERAPY INDUCES A SIGNIFICANT UPREGULATION OF THE PLASMA LIPIDOME IN FH PATIENTS

Reijo Laaksonen[1], M. Jänis[1], K. Tarasov[1], R. Hurme[1], D. Marais[2], K. Ekroos[1]

[1]Zora Biosciences Oy, Espoo, Finland, [2]University of Cape Town, Cape Town, South Africa

Objectives: We characterized the effect of atorvastatin ± torcetrapib on plasma lipidome in familial hypercholesterolemia (FH) patients.

Methods: Samples were analyzed by mass spectrometry based lipidomics. The samples were from a randomized trial including FH patients on atorvastatin (80 mg/day; n=16), and atorvastatin 80 mg plus torcetrapib (n=16).

Results: The analyses revealed that the lipid treatment lowered significantly plasma cholesteryl ester (CE) species. In the atorvastatin group CE 20:4 concentration was lowered by -28% ($p < 0.05$) and in the combination treatment group CE 19:0, 19:2, 20:0,21:0 22:3 and 24:0 were reduced significantly from -15% to -29%. Despite decreased CE concentrations most of the other lipid species were upregulated elevated in treated FH patients. In the atorvastatin group significantly elevated (from +18% to 43%) concentrations of lysophosphatidylcholine (LPC) 18:0, LPC 18:1, CE 20:0, ClcCer glucosylceramide (GlcCer) d18:1/24:0, GClcuCer d18:1/22:0 and ether-linked phosphatidylcholine (PC-O)0 38:1 were recorded. In the combination group the significantly increased (+15-61%) lipid species included: sphingomyelin (SM) (d18:1/17:0), (18:1/16:1-OH), SM (18:1/17:0)(18:1/15:2-OH), SM (18:1/23:0), (18:1/22:1-OH)LPC 18:2, ceramide (Cer) d18:1/24:1, phosphatidylcholine (PC) 18:0/20:4, PC O-38:7, PC O-32:1, PC O-34:2, PC O-38:4, PC O-34:3, PC O-36:1. Overall we recorded decreased plasma concentrations only for 13% and 28% of all detected lipid species during atorvastatin and atorvastatin-torcetrapib treatments respectively.

Conclusions: Lipidomic analyses unraveled an atypical lipidomic profile response in FH patients due to lipid lowering treatment. Inhibition of cholesterol synthesis in FH patients may result in compensatory hepatic synthesis of a number of lipids to compensate the reduced LDL-receptor mediated lipid uptake.

Workshop: UPTAKE, STORAGE AND MOBILIZATION OF LIPIDS AND CELLULAR CONSEQUENCES

ATGL-DEFICIENT MICE OVEREXPRESSING ATGL IN HEART ARE RESISTANT TO DIET-INDUCED OBESITY AND INSULIN RESISTANCE

Renate Schreiber, R. Zimmermann, G. Haemmerle, R. Zechner

Institute of Molecular Biosciences, University of Graz, Graz, Austria

Insulin resistance is associated with elevated free fatty acid (FFA) levels in the circulation. Plasma FFA levels are predominantly determined by adipose tissue lipolysis and the rate-limiting enzyme in triacylglycerol (TG) mobilization is adipose triglyceride lipase (ATGL). ATGL-deficient (ATGL-/-) mice show decreased FFA levels, enhanced glucose usage, and improved insulin sensitivity. These observations link ATGL function to the pathogenesis of type 2 diabetes and identify ATGL as a potential drug target for the treatment of this disease. However, ATGL-/- mice also showed a massive TG accumulation in cardiac muscle leading to premature death. This severe phenotype makes it difficult to investigate whether ATGL-deficiency counteracts the development of diet-induced insulin resistance.

The aim of this study is to investigate energy metabolism in ATGL-deficient mice overexpressing ATGL specifically in heart (heartATGL-/- mice). Plasma lipid parameters and glucose levels were similar in ATGL-/- mice and heartATGL-/- mice. Yet, heartATGL-/- mice did not accumulate TG in cardiac muscle and exhibited normal life expectancy. To study ATGL function during high caloric intake, mice were fed a high-fat diet for 2 months. Compared to controls, plasma TG and FFA levels were decreased by 50% and 60%, respectively. Blood glucose levels were decreased by 50 % indicating resistance of these animals to diet-induced type 2 diabetes. Insulin sensitivity tests showed that heartATGL-/- mice retain improved insulin sensitivity even on a high-fat diet. In conclusion, our data demonstrate that heartATGL-/- mice are resistant to the development of diet-induced insulin resistance and type 2 diabetes.

Workshop: UPTAKE, STORAGE AND MOBILIZATION OF LIPIDS AND CELLULAR CONSEQUENCES

BROWN ADIPOSE TISSUE IS A MAJOR DETERMINANT OF PLASMA CLEARANCE AND ORGAN UPTAKE OF TRIGLYCERIDE-RICH LIPOPROTEINS

Alexander Bartelt[1], O.T. Bruns[1,2], K. Peldschus[3], H. Ittrich[3], U.I. Tromsdorf[4], M. Merkel[1,5], J. Heeren[1]

[1]IBMII: Molecular Cell Biology, N30, University Medical Center Hamburg-Eppendorf, [2]Electronmicroscopy and Micro-Technology, Heinrich-Pette-Institute for Experimental Virology and Immunology, [3]Department of Diagnostic and Interventional Radiology, University Medical Center Hamburg-Eppendorf, [4]Department of Physical Chemistry, University of Hamburg, [5]1. Medizinische Klinik, Asklepios Klinik St. Georg, Hamburg, Germany

Objective: Chylomicrons and VLDL represent triglyceride-rich lipoproteins (TRL) that transport triglycerides to peripheral tissue for storage and energy supply. Recently it has been shown that adults have substantial amounts of functional brown adipose tissue (BAT). Murine as well as human BAT has a high energy demand especially when it is activated upon cold exposure. Here we investigate the role of TRL metabolism for energy supply of BAT upon cold exposure in mice.

Methods: C57BL/6J mice were kept for 24 hours in a cold room (8°C) or at room temperature. mRNA expression levels, plasma clearance and organ uptake of radiolabelled TRL were determined in control and cold-adapted mice. TRL organ uptake was visualised by magnetic resonance imaging (MRI) using nanocrystals embedded into the TRL core.

Results: In BAT cold exposure increases expression of genes required for catabolism (e.g. LPL and apoE). In line with these findings we observed a massive acceleration of TRL turnover and a concomitant 20-fold increase in organ uptake of TRL components into BAT of cold-adapted mice. Using MRI we visualised the dynamic uptake of TRL into BAT and liver in control as well as in cold-adapted mice. Taken together, we observe an accelerated TRL plasma clearance with an enormous shift in TRL uptake from liver to BAT in cold-adapted mice.

Conclusion: BAT is a novel determinant of plasma clearance and organ uptake of TRL especially upon cold exposure. Therefore, BAT may be an interesting target in diseases associated with elevated serum triglycerides such as diabetic dyslipidemia.

Workshop: UPTAKE, STORAGE AND MOBILIZATION OF LIPIDS AND CELLULAR CONSEQUENCES

GLUCAGON-LIKE PEPTIDES (GLP-1 AND GLP-2) AS CRITICAL REGULATORS OF INTESTINAL LIPID ABSORPTION AND CHYLOMICRON PRODUCTION

J. Hsieh[1], **Khosrow Adeli**[2]

[1]University of Toronto, [2]Clinical Biochemistry, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Many peptide hormones produced within the gastrointestinal system aid in the regulation of energy homeostasis and metabolism. Among these are glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). Recent studies in our laboratory indicate that GLP-1 has an inhibitory effect on intestinal apoB-containing lipoprotein output in the Syrian Golden hamster, but the effect is lost in fructose-fed, insulin-resistant hamsters, suggesting that insulin signaling is crucial for GLP-1's action on the intestine. We found that the GLP-1 agonist exendin-4 has a significant acute suppressive effect on intestinal fat absorption and CM production in the Syrian Golden hamster. In addition, intraperitoneal administration of the GLP-1 antagonist exendin (9-39) resulted in significantly greater CM secretion than the control. GLP-1's suppressive effect on CM assembly and secretion may in part be mediated through its insulinotropic effect, since fructose fed hamsters that do not respond to insulin exhibited no change in intestinal lipoprotein secretion with administration of GLP-1. In contrast, GLP-2 showed a marked stimulatory effect on CM secretion in both mice and hamster, consistent with its general role in facilitating nutrient absorption. Moreover, GLP-2 directly stimulated apoB48 secretion in jejunal fragments cultured ex vivo, increased expression of fully glycosylated CD36 and induced intestinal absorption of [^{3}H]triolein. Similarly, GLP-2 significantly enhanced intestinal lipoprotein production in wild-type mice but not in *Cd36*$^{-/-}$ mice. Thus, GLP-2 acts as a potent stimulator of intestinal CM secretion possibly through enhanced lipid uptake in a pathway requiring CD36.

Workshop: HDL - MORE THAN CHOLESTEROL?

HIGH DENSITY LIPOPROTEIN PROTECTION AGAINST PRIMARY CARDIAC RISK IS LOST WITH ENHANCED LOW GRADE CHRONIC INFLAMMATION

Robin Dullaart[1], R. Gansevoort[2], C. Sparks[3], J. Corsetti[4]

[1]Endocrinology, [2]Nephrology, University of Groningen, Groningen, The Netherlands, [3]Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, [4]Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Background: HDL-cholesterol predicts recurrent cardiovascular risk in a subgroup of postinfarction patients defined by hypercholesterolemia and high C-reactive protein levels. We investigated whether a similar high-risk subgroup exists for incident cardiovascular disease in a population-based cohort..

Subjects and methods: A graphical exploratory data analysis tool was used to identify high-risk subgroups in a male population-based cohort (n = 3405) from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study by generating 3-dimensional mappings of risk over the HDL-cholesterol/C-reactive protein domain with subsequent use of Kaplan-Meier analysis to verify high-risk. Within-subgroup risk was assessed using Cox proportional hazards regression and Kaplan-Meier analysis.

Results: Mappings revealed two high-risk subgroups: a low HDL-cholesterol/high C-reactive protein subgroup and a smaller high HDL-cholesterol/high C-reactive protein subgroup. The low HDL-cholesterol subgroup demonstrated a pattern of metabolic syndrome-like dyslipidemia in contrast with a predominantly unremarkable biomarker pattern for the high HDL-cholesterol subgroup. However, in the high HDL-cholesterol subgroup, C-reactive protein levels were higher than in the low HDL-cholesterol subgroup. Within the high HDL-cholesterol subgroup, C-reactive protein predicted risk. Moreover, in the high HDL-cholesterol subgroup, risk was associated with lower triglyceride levels in conjunction with presumptively larger HDL particles.

Conclusions: High HDL-cholesterol and high C-reactive protein levels define a subgroup of men at high-risk for incident cardiovascular disease. High HDL cholesterol-associated risk likely relates to impaired anti-inflammatory properties of HDL particles remodeling. This approach may facilitate identification of abnormal HDL function-related properties conferring high risk, despite high HDL-cholesterol associated risk.

Workshop: HDL - MORE THAN CHOLESTEROL?

A NOVEL METHOD FOR THE MEASUREMENT OF PREBHDL AND AHDL KINETICS WITH STABLE ISOTOPIC LABELLING TECHNIQUES

Xuefei Li, M. Stolinski, N.C. Jackson, M.A. Umpleby

Diabetes and Endocrinology, PGMS/University of Surrey, Guildford, UK

Aim: Current understanding of HDL kinetics is limited due to an inability to measure the kinetics of preβHDL. The aim of this study was to develop a method to determine the kinetics of apoA-I in αHDL and preβHDL following isotopic labelling.

Methods: A novel technique to isolate apoA-I from αHDL and preβHDL was developed. Following an overnight fast, six healthy subjects (4F+2M) received a 9 hour intravenous infusion of 1-^{13}C-leucine with hourly blood sampling. ApoA-I from plasma αHDL and preβHDL was separated by a combination of agarose gel electrophoresis and SDS-PAGE. HDL_2 and HDL_3 were also separated by ultracentrifugation and apoA-I isolated by SDS-PAGE. ApoA-I bands were hydrolyzed in 6M HCl, amino acids purified by ion exchange chromatography and isotopic enrichment measured by GCMS. The fractional catabolic rate (FCR) of HDL apoA-I was calculated using the rate of increase of apoA-I enrichment and the steady state enrichment of α-ketoisocaproate.

Results: Plasma cholesterol was 4.42±0.34 mmol/L (mean±SEM), triglyceride 0.91±0.56 mmol/L, HDL cholesterol 1.07±0.10 mmol/L and total plasma apoA-I 1.32±0.09 mg/ml. PreβHDL apoA-I FCR was significantly higher than αHDL apoA-I FCR, 0.20±0.02 vs 0.17±0.02 pools/day respectively (*P*=0.007). There was a significant negative correlation between preβHDL FCR and plasma apoA-I and between αHDL FCR and plasma apoA-I (both p=0.008). There was also a significant difference between HDL_2 and HDL_3 FCR, 0.18±0.02 vs 0.16±0.01 pools/day respectively (*P*=0.003).

Conclusion: Results indicate αHDL and preβHDL kinetics can be measured by this methodology. This technique may aid our understanding of the mechanisms which cause reduced HDL cholesterol.

Workshop: ATHEROTHROMBOSIS

C HANGES OF PROINFLAMMATORY CYTOKINES, CHEMOATTRACTANTS AND DESTRUCTIVE METALLOPROTEINASES CONTENTS DURING OF UNSTABLE ATHEROSCLEROTIC PLAQUE FORMATION

Yulia Ragino[1], A. Chernjavski[2], Y. Polonskaja[1], A. Volkov[2], E. Semaeva[2], S. Tcimbal[2], M. Voevoda[1]

[1]Institute of Internal Medicine SB RAMS, [2]Research State of Circulation Pathology, Federal Agency of Public Health and Social Development, Novosibirsk, Russia

Inflammatory biomarkers (TNF-alpha, IL-1-beta, IL-6, IL-8, high sensitive C-reactive protein, hsCRP, monocytes chemotactic protein, MCP-1, endothelial monocytes activating protein, EMAP-II, adhesive molecules ICAM-1 and VCAM-1) and destructive biomarkers (metalloproteinase, MMP-3, MMP-7, MMP-9, tissue inhibitor of metalloproteinase, TIMP-1) during of consecutive stages of atherosclerotic plaque formation (normal intimae, lipid stain, early stable plaque, unstable vulnerable plaque, stable plaque with fibrosis") and in 3 types of plaques instability (lipid, inflammatory-erosive and necrotic) were studied. The study was included 96 men with angiographic proven coronary atherosclerosis. Collecting of 162 intima/media fragments was performed during coronary artery bypass grafting operation by endarterectomy. After histological analysis of fragments the "normal intimae" was revealed in 19 cases, "lipid stain" - in 23, "early stable plaque" - in 34, "stable plaque with fibrosis" - in 41, "unstable plaque" - in 45, included lipid type in 14 cases, inflammatory-erosive type - in 16 and necrotic type - in 15. Biomarkers in homogenate of fragments were investigated by ELISAs kits after protein measuring. Increased levels of IL-6, IL-8, hsCRP, MCP-1 and EMAP-II as inflammatory biomarkers, on the one hand, and increased levels of MMP-7 and MMP-9 as destructive biomarkers, on the other hand, were typical for unstable plaques. Inflammatory activity was prevailed not only in inflammatory-erosive type, but in lipid type of unstable plaques in comparison with necrotic type. Thus, inflammatory-destructive activity changes are significant for unstable plaque formation and they are different in various types of plaques instability. The study was supported by Grant of RFBI 09-04-00374.

Workshop: ATHEROTHROMBOSIS

FVIIA PLASMA LEVELS: INFLUENCE OF F7, F10 AND PROCR (ENDOTHELIAL PROTEIN C RECEPTOR) SNPS

Inge Kalies[1], M. Knutsson[1], A. Siegbahn[2], A.-C. Syvänen[2], L. Lind[2], R. Kerb[1,3]

[1]AstraZeneca R&D, Mölndal, [2]Medical Science, Uppsala University, Uppsala, Sweden, [3]Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany

Objective: Investigation on the influence of candidate single nucleotide polymorphisms (SNPs) on plasma levels of Coagulation factor FVIIa. FVII/FVIIa levels are consistently and independently related to cardiovascular risk and their high variability in plasma level/activity may be partially due to genetic factors.

Population: A population-based sample of ~1000 subjects (70-yrs-old, 51% men) from the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS).

Methods: 155 SNPs from 20 genes, reported to influence blood coagulation and fibrinolysis were selected from the database (http://www.ncbi.nml.nih.gov/SNP/). Genotyping using the Golden Gate assay (Illumina Inc.) was done at the SNP technology platform Uppsala University. Plasma levels of active PAI-1, t-PA:ag, vWF:ag, F1+2, fibrinogen, D-dimer and FVIIa were analyzed at the Uppsala Clinical Research laboratory (UCR).

Results: Four F7 SNPs were found associated to circulating FVIIa levels (p: E-55 to E-59). Three vWF SNPs showed association to vWF:ag (p: E-7 to E-8). Furthermore, six F10 SNPs (p: E-6 to E-46) and the PROCR SNP rs2069940 (p=E-9) correlated with FVIIa level. F7 and F10 genes are localized in close proximity on chromosome 13q with the most significant F10 SNP (rs474810) in strong LD with the F7 SNP rs6046 (R353Q), strongly influencing FVIIa levels. The PROCR SNP rs2069940 belongs to the A3 haplotype, recently associated with the amount of soluble EPCR (sEPCR) in plasma that binds FVIIa (Ireland, atvb09).

Conclusion: Our results support the role of EPCR and FVIIa as risk factors for coronary heart disease, confirming the existence of complex dynamic processes linking FVII, FX and EPCR.

Workshop: ATHEROTHROMBOSIS

CAN ECCENTRIC ARTERIAL PLAQUES ALONE CAUSE FLOW STAGNATION POINTS AND FAVOUR THROMBUS INCORPORATION?

Simone G. Ramos[1], C.T. Beneli[1], P.F. Barbosa[1], E.M. Floriano[1], J. Elias Jr[2], F.S. Ramalho[1], M.A. Rossi[1]

[1]Pathology, [2]Radiology, Faculty of Medicine of Ribeirão Preto/University of São Paulo, Ribeirão Preto, Brazil

Introduction: Many studies of human arteries have confirmed that various stages of thrombosis and thrombus incorporation can be involved in the process of plaque progression. This is many times associated with heart attack and stroke, the most frequent causes of the morbidity and mortality in atherosclerosis. A majority of studies pertaining to this subject refer to thrombosis as being related to vulnerable lipid plaques or endothelial injury after surgical proceedings, exposing tissue factor and triggering the coagulation cascade.

Objectives: To determine whether eccentric atherosclerotic plaques can alone cause haemodynamic alterations, resulting in flow stagnation points that favour thrombosis as well as incorporation, contributing to a continuous growing of the atheroma. The research presented here was undertaken to test this hypothesis.

Method: An experimental model of stenosis of the aorta was used with a mushroom-shaped Plexiglas plug, simulating a stable atheromatous plaque and promoting local turbulence and thrombosis. This type of stenosis, named intrinsic or intraluminal, minimizes possible intramural effects associated with external compression or ligation, which severely deform the arterial wall.

Results and conclusion: With animal survival of more than 24 h, we followed the partial fibrinolysis of the thrombus as well as its posterior organization and incorporation to the arterial wall as a neointima for up to 30 days. The mushroom plug form permitted the development of recirculation and stasis areas around it, favoring this evolution. Despite noted limitations, this study demonstrates that thrombus incorporation can contribute to plaque extension, as it can promote recirculation and stasis areas.

Workshop: ATHEROTHROMBOSIS

SIGNIFICANT ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN VITAMIN K EPOXIDE REDUCTASE GENE AND THE RISK OF ISCHEMIC STROKE

Fang-I Hsieh[1,2], Y.-C. Hsieh[1], C.-W. Chang[1], C.-H. Bai[1,3], L.-M. Lien[4], J.-S. Jeng[5], H.-J. Lin[6], H.-P. Tseng[7], W.-C. Lo[1], Y.-L. Li[1], H.-Y. Chiou[1,2]

[1]School of Public Health, Taipei Medical University, [2]Dr. Chi-Chin Huang Stroke Research Center, Taipei Medical University, [3]Central Laboratory, [4]Department of Neurology, Shin Kong Wu Ho-Su Memorial Hospital, [5]Stroke Center and Department of Neurology, National Taiwan University Hospital, [6]Department of Neurology, Chi-Mei Medical Center, [7]Department of Neurology, Lotung Poh-Ai Hospital, Taipei, Taiwan R.O.C.

Background: The formation of thrombus has been confirmed to play an important role for the development of ischemic stroke. In addition, the contribution of the coagulation cascade to the initiation and propagation of the thrombus has been reported in many studies. Several studies indicated that the formation of coagulation factors was associated to the Vitamin K cycle. The important step in this cycle was conversion of vitamin K to vitamin K epoxide catalyzed by vitamin K epoxide reductase (VKOR). Therefore, we hypothesize that polymorphisms in the VKOR gene are associated with the risk of ischemic stroke.

Methods: The case-control study was conducted with 701 ischemic stroke patients and 701 controls. All cases were confirmed with computed tomography and/or magnetic resonance imaging. A structured questionnaire was used to collect information on conventional cardiovascular risk factors and laboratory results. The genetic polymorphisms of VKOR (A-1639G, T1173C and T2255C) were determined by polymerase chain reaction-restriction fragments length polymorphism.

Results: A significantly increased risk of ischemic stroke was found in the subjects with VKOR -1639G/1173C/2255C carriers (OR=1.82, 95% C.I.=1.21-2.74) as compared with those without these mutations after adjusted for hypertension, dyslipidemia, diabetes, history of cardiovascular diseases, and family history of stroke. Moreover, a drastically elevated risk of ischemic stroke (OR=11.26, 95% C.I.=4.77-26.56) was observed in subjects with diabetes and GCC haplotype compared to those without these two risk factors.

Conclusion: The GCC haplotype of VKOR gene was a strong predictor of ischemic stroke especially for diabetes patients.

Workshop: LIPID MEDIATORS OF INFLAMMATION AND CELL SIGNALING

TIMP3: A SWITCH THAT CONTROLS HIGH FAT DIET-INDUCED INFLAMMATION, DIABETES AND STEATOSIS IN MICE

Rossella Menghini[1], V. Casagrande[1], M. Cavalera[1], V. Marzano[1], S. Menini[2], G. Pugliese[2], A. Urbani[1], R. Lauro[1], M. Federici[1]

[1]*University of Rome Tor Vergata,* [2]*University of Rome La Sapienza, Rome, Italy*

Obesity is associated with low-grade inflammatory state which prompts to diabetes and atherosclerosis. We found that activity and expression of Tissue inhibitor of Metalloproteinase 3 (TIMP3) are decreased in obesity. Because obesity is associated with increased macrophage accumulation in different tissues, we used a transgenic approach under control of CD68 promoter to reconstitute TIMP3 levels and we analyzed its metabolic homeostasis. After 20 weeks of High Fat Diet (HFD), we observed that Tg mice compared with WT showed a significant improved glucose tolerance and insulin sensitivity, measured by IPGTT and IPITT ($p< 0.01$ for all, $n=8$ per group). Histology of white adipose tissue (WAT), liver, kidney and aorta showed accumulation of lipids, intense fibrosis and increased inflammatory markers in WT compared with Tg mice. Expression profiling for metabolic and inflammatory genes revealed that Tg compared with WT have significant lower levels for IL6, SOCS3 and SCD1 in WAT, and for Socs3, IL-6, G6Pase in liver ($p< 0.01$ for all, $n=5$). To further elucidate the mechanisms involved in Timp3 rescue of hepatic steatosis we analyzed liver homogenates by shotgun proteomics (ESI-LC-qTOF-MS/MS) identifying 62 differentially expressed proteins in WT compared with Tg mice, including ACLS-1 (5-fold higher in WT vs Tg, $p< 0.001$) and FABP-1 (2.2 higher in WT vs Tg, $p< 0.01$), which were confirmed by western blot. Our data indicate that macrophage specific overexpression of TIMP3 protects from HFD induced inflammation and metabolic disorders.

Workshop: LIPID MEDIATORS OF INFLAMMATION AND CELL SIGNALING

GENETIC VARIATION IN LIVER X RECEPTOR ALPHA AND RISK OF ISCHEMIC VASCULAR DISEASE IN THE GENERAL POPULATION

Stefan Stender[1], R. Frikke-Schmidt[1], A.A. Sethi[2], B.G. Nordestgaard[3], A. Tybjærg-Hansen[1]

[1]Dept. Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [2]Lipoprotein Metabolism Section, Vascular Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA, [3]Dept. Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark

Background and aims: Liver X receptor alpha (LXRα) is a nuclear receptor that plays important roles in both lipid metabolism and inflammation. We hypothesized that genetic variation in LXRα could influence plasma lipid and lipoprotein levels and risk of ischemic vascular disease in the general population.

Methods: We genotyped seven LXRα single nucleotide polymorphisms (SNPs) in the Copenhagen City Heart Study, a prospective general population study comprising a total of 10,300 subjects with 33 years of follow-up. We subsequently examined plasma lipid and lipoprotein levels and risks of ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease and ischemic stroke as a function of LXRα genotype.

Results: Homozygosity for -840C>A and -115G>A (in perfect linkage disequilibrium), present in 3%, associated with a hazard ratio (HR) for ischemic heart disease of 1.3 (95% confidence interval [CI], 1.0-1.7; P=0.04), a HR for myocardial infarction of 1.6 (95% CI, 1.1-2.2; P=0.008), a HR for ischemic cerebrovascular disease of 1.6 (95% CI, 1.2-2.2; P=0.002) and a HR for ischemic stroke of 1.8 (95% CI, 1.3-2.6; P< 0.001). Heterozygosity for -840C>A and -115G>A did not associate with any disease outcomes. Furthermore, none of the seven genotyped SNPs associated with plasma lipid and lipoprotein levels.

Conclusion: Homozygosity for LXRα -840C>A and -115G>A associated with increased risks of ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease and ischemic stroke in the general population.

Workshop: LIPID MEDIATORS OF INFLAMMATION AND CELL SIGNALING

EXPRESSION OF A RETINOIC ACID SIGNATURE IN CIRCULATING CD34 CELLS FROM CORONARY ARTERY DISEASE PATIENTS

Tineke CTM van der Pouw Kraan[1], S.H. Schirmer[2], J.O. Fledderus[3], P.D. Moerland[3], J.M. Baggen[1], T.A. Leyen[1], A.M. van der Laan[3], J.J. Piek[3], N. van Royen[3], A.J.G. Horrevoets[1]

[1]VU University Medical Center, Amsterdam, The Netherlands, [2]Universitätsklinikum des Saarlandes, Homburg, Germany, [3]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction: Circulating CD34+ progenitor cells have the potential to differentiate into a variety of cells, including endothelial cells. Knowledge is still scarce about the transcriptional programs used by CD34+ cells from peripheral blood, and how these are affected in coronary artery disease (CAD) patients.

Methods: We performed a whole genome transcriptome analysis of CD34+ cells, CD4+ T cells, CD14+ monocytes, and macrophages from 12 patients with CAD and 11 matched controls.

Results: CD34+ cells, compared to other mononuclear cells from the same individuals, showed high levels of KRAB box transcription factors, known to be involved in gene silencing. This correlated with high expression levels in CD34+ cells for the progenitor markers HOXA5 and HOXA9, which are known to control expression of KRAB factor genes. The comparison of expression profiles of CD34+ cells from CAD patients and controls revealed a less naïve phenotype in patients' CD34+ cells, with increased expression of genes from the Mitogen Activated Kinase network and a lowered expression of a panel of histone genes, reaching levels comparable to that in more differentiated circulating cells. Furthermore, we observed a reduced expression of several genes involved in CXCR4-signalling and migration to SDF1/CXCL12. The altered gene expression profile of CD34+ cells in CAD patients was related to activation/differentiation by a retinoic acid-induced differentiation program.

Conclusion: These results suggest that circulating CD34+ cells in CAD patients are programmed by retinoic acid, leading to a more differentiated phenotype and reduced capacity to migrate to ischemic tissues.

Workshop: LIPID MEDIATORS OF INFLAMMATION AND CELL SIGNALING

FXR-DEFICIENCY IMPROVES GLUCOSE AND ENERGY HOMEOSTASIS IN GENETIC OBESITY

Janne Prawitt[1], M. Abdelkarim[1], J. Dumont[1], H. Duez[1], J.H.M. Stroeve[2], T.H. van Dijk[2], E. Bouchaert[1], F. Kuipers[2], B. Cariou[1], S. Caron[1], B. Staels[1]

[1]Departement d'Atherosclerose, Institut Pasteur de Lille, Univ Lille Nord de France, Inserm U545, UDSL, Lille, France, [2]Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands

Introduction: Obesity predisposes to the development of insulin resistance and type 2 diabetes with a subsequent increase in the risk for atherosclerosis. The underlying molecular links between these pathologies are not very well understood. The nuclear receptor FXR has lately been established as a regulator of glucose homeostasis, insulin sensitivity and energy metabolism in lean mice.

Objective: To study the role of FXR in an obese context, we analysed the effects of FXR deficiency in genetic obesity, using $FXR^{-/-}$ob/ob mice.

Results: Obesity was attenuated in $FXR^{-/-}$ob/ob mice compared to $FXR^{+/+}$ob/ob controls reflected in reduced fat and lean mass, probably due to a decrease in energy balance. Glucose homeostasis was strongly improved in $FXR^{-/-}$ob/ob mice as detected by a reduction of hyperglycemia and hyperinsulinemia and a clearly ameliorated glucose tolerance assessed in both IPGTT and OGTT. The increase of the metabolic clearance rate of blood glucose measured by stable isotope dilution and an enhanced Akt-phosphorylation in response to an acute insulin stimulus in white adipose tissue and skeletal muscle but not in liver of $FXR^{-/-}$ob/ob mice strongly indicated an amelioration of peripheral insulin sensitivity. Thus, the observed decrease in hepatic gluconeogenic gene expression in $FXR^{-/-}$ob/ob mice seems to be due to a direct effect of FXR-deficiency, and might well contribute to the reduction of blood glucose levels.

Conclusion: Our data show an unexpected role of FXR in the development of obesity and related disturbances in glucose homeostasis.

Workshop: PLAQUE PROGRESSION AND RUPTURE

MICE DOUBLE-DEFICIENT FOR APOLIPOPROTEIN E AND BIGLYCAN SHOW ENHANCED ATHEROSCLEROSIS ON NORMAL CHOW

Ariane Melchior-Becker, J.W. Fischer

Pharmokologie, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, Germany

The small leucin-rich repeat proteoglycan biglycan is postulated to play on one hand a key role in collagen matrix assembly and on the other hand in lipid retention due to its LDL-binding properties. To analyse the functional role of biglycan in atherogenesis we bred male mice double deficient of apolipoproteinE and biglycan (ApoE-/-/bgn-/0) and analysed the progression of atherosclerosis compared to ApoE-deficient littermates (ApoE-/-/bgn+/0) at 15, 30 and 35 weeks on normal chow. ApoE-/-/bgn-/0-mice showed progressive dilatation of the aortic arch in comparison to age matched littermates. Bodyweight increased significantly during ageing in ApoE-/-/bgn-/0 but not in ApoE-/--littermates. ApoE-/-/bgn-/0-mice showed dramatically increased Plaque burden as determined by oil-red O en face staining of the thoracic aorta at all ages. In addition, plaque area of aortic root lesions showed enlarged lesions in ApoE-/-/bgn-/0-mice at the age of 30 and 35 weeks. While there were no differences found in lipid content of the aortic root plaques, the collagen content differed as well as the collagen fibril density as evidenced by sirius red staining and analysis under polarized light. ApoE-/-/bgn-/0-mice showed decreased accumulation of hyaluronan and a trend towards increased accumulation of decorin in aortic root lesions. Furthermore, ApoE-/-bgn-/0 mice were characterized by dramatically increased macrophage retention in aortic root lesions at 15 weeks. Taken together ApoE-/-/bgn-/0-mice showed increased atherosclerosis compared to their ApoE-/--littermates on normal chow possibly due to increased macrophage mediated inflammation and/or disturbed vascular collagen matrix. These results strongly suggest a protective role of biglycan during the progression of atheroslerosis.

Workshop: PLAQUE PROGRESSION AND RUPTURE

EFFECT OF GDF-15 (GROWTH DIFFERENTIATION FACTOR 15) IN THE PROGRESSION OF ATHEROSCLEROSIS

Stefanie Zügel[1], S. Vorwald[2], G. Bendner[2], G.A. Bonaterra[1], J. Strelau[2], R. Kinscherf[1]

[1]Anatomy and Cell Biology, Philipps-University, Marburg, [2]Anatomy and Cell Biology III, Ruprecht-Karls University, Heidelberg, Germany

Growth differentiation factor-15 (GDF-15) is a member of the TGF-ß superfamily and an important regulator of proliferation, differentiation and inflammatory processes and found in human atherosclerotic plaque. Hence the goal of the study was to investigate the effect of GDF-15 in the progression of atherosclerosis in apoE deficicent (apoE$^{-/-}$) mice. Therefore GDF-15 deficient (GDF-15$^{-/-}$) LacZ-knockin mice were generated and crossbred with apoE deficient mice. At an age of 10 weeks, the offspring were fed with a high cholesterol diet (western diet) for a period of 12 weeks. The extent of atherosclerotic plaque was measured in the innominate artery (=brachiocephalic trunk). Detection of BM8 and MOMA-2 (macrophages), Ki67 (proliferation), COX-2 and MIF (inflammation) was performed via immunohistological staining and computer-assistedly measured. In apoE$^{-/-}$/GDF-15$^{-/-}$ mice we found a reduction of lumen stenosis of 37% (p=0.05) compared to apoE$^{-/-}$/GDF-15$^{+/+}$ mice. Expression of COX-2 (+91%, p=0.02) and MIF (+202%, p=0.04) was increased and although Ki67 positive cells were increased (+94%, p=0.05) no change in BM8 and MOMA-2 positive cells was detected. We assume that an increased proliferation and inflammatory reactions in atherosclerotic lesions of apoE$^{-/-}$/GDF-15$^{-/-}$ mice indicate an important role of GDF-15 in the progression of atherosclerosis.

Workshop: PLAQUE PROGRESSION AND RUPTURE

GENETIC DEFICIENCY IN FUNCTIONAL MMP-10 REDUCES PROGRESSION OF ATHEROSCLEROSIS IN APOLIPOPROTEIN E-KNOCKOUT MICE

Ana Purroy, M. Belzunce, J. Orbe, J. Barrenetxe, J.A. Páramo, J.A. Rodríguez

Atherosclerosis Research Laboratory, Division of Cardiovascular Sciencies, Centre for Applied Medical Research (CIMA). University of Navarra, Pamplona, Spain

Objective: Matrix metalloproteinases (MMPs) participate in different stages of atherothrombosis. MMP-10 is expressed in human atherosclerotic lesion, but its role in atherosclerosis remains poorly understood. We have assessed the relevance of MMP-10 on atherosclerosis by determining the impact of MMP-10 deficiency in the apolipoprotein E knockout (apoE-/-) mice.

Methods: Aortic MMP-10 expression was measured in wild type (wt) and apoE-/- mice by RT-qPCR. ApoE-/- (n=16) and double knockout (apoE-/-;MMP-10-/-) mice (n=20) on C57Bl6J background were kept on normal chow diet until sacrifice (10 and 20 months). Morphometric analysis were performed on serial sections of brachiocephalic artery and aortic root. Collagen content (Sirius red staining), plaque composition (smooth muscle and macrophages) and localization of MMP-10 expression by immunohistochemistry were measured.

Results: Aortic MMP-10 expression was observed in the atherosclerotic lesion of apoE-/- but was not in wt mice, colocalizing by immunohistochemistry with macrophages in the core and shoulders of the plaque. Plaque size at 10 months was reduced up to 70% ($p< 0.001$) in the brachiocephalic artery and aortic root of apoE-/-;MMP-10-/- as compared to apoE-/- mice. A 40% reduction ($p< 0.05$) was also observed in older animals. Frequency of calcified plaques at 10 months was significantly decreased in apoE-/-;MMP-10-/- mice (85 vs 33%, $p< 0.05$), but not at 20 months.

Conclusion: Our study provides in vivo evidence that MMP-10 plays a major role in atherosclerosis progression and participates in plaque calcification.

Workshop: PLAQUE PROGRESSION AND RUPTURE

GEOMETRY OF THE VULNERABLE PLAQUE: LONGITUDINAL ASYMMETRY IS PREDICTIVE FOR RUPTURE

Iwona Cicha[1], K. Beronov[2], M. Klein[3], D. Raithel[3], W.G. Daniel[1], C.D. Garlichs[1]

[1]Department of Cardiology and Angiology, University of Erlangen-Nuremberg, Erlangen, [2]Leibnitz Supercomputing Centre, Bavarian Academy of Sciences, Munich, [3]Department of Vascular Surgery, Clinic South Nuremberg, Nuremberg, Germany

Objectives: Atherosclerotic plaque rupture, the main cause of acute coronary syndromes and ischemic strokes, occurs predominantly at the upstream region. In this study, we performed longitudinal analysis of the structural aspects of plaque morphology.

Methods: Plaque length, total intima thickness, lipid core thickness, and fibrous cap (FC) thickness were measured at 5 points along 15 upstream-ruptured and 15 non-ruptured stable plaques. Averaged geometric profiles outlining the outer shapes and lipid core shapes for ruptured and stable plaques were constructed.

Results: There were no significant differences in plaque length between upstream-ruptured and non-ruptured plaques. However, striking differences in plaque geometry were detected between these plaques. Compared with stable plaques, upstream-ruptured plaques exhibited a significantly thinner FC at their upstream shoulder, whereas FC thickness at the downstream shoulder was comparable. For ruptured plaques, the lipid core upstream amounted to 83% of plaque thickness, which was significantly higher than the values obtained for stable plaques (49%, $P< 0.001$).

Compared with stable plaques, which were largely symmetric, ruptured plaques exhibited a strongly pronounced asymmetry. FC thickness upstream of the ruptured plaques was significantly smaller (65µm) than at the downstream side (546µm; $P< 0.001$), and the lipid core thickness significantly bigger ($P< 0.001$).

Conclusions: Vulnerability of the atherosclerotic plaque is reflected in the asymmetry of the FC thickness and of the lipid core shape. Comparison of the ratio of soft to harder components between the upstream and downstream region of a suspect plaque should allow an earlier detection of plaques at risk of rupture.

Workshop: FAT DEPOTS, INSULIN RESISTANCE AND ATHEROSCLEROSIS

VISCERAL OBESITY CAUSED BY OVEREXPRESSING SREBP-1C IN LIVER

B. Knebel[1], J. Haas[2], S. Jacob[1], U. Nitzgen[1], **Dirk Müller-Wieland**[3], J. Kotzka[1]

[1]German Diabetes Center, Düsseldorf, [2]Asklepios Clinic St. George, [3]I. Med., Abteilung für Innere Medizin, Asklepios Klinik St. Georg, Hamburg, Germany

Objective: Sterol regulatory element binding protein (SREBP)-1c is a key transcription factor that mediates gene regulatory effects of hormones, cytokines, and metabolites.

Methods: The systemic impact of this central regulator of lipid metabolism in vivo was analyzed in transgenic mouse models expressing the N-terminal transcriptional active domain of SREBP-1c tissue specifically in liver and adipose tissue, respectively.

Results: Animals were kept under standardized conditions with regular chow until the age of 24 weeks. Overexpression of SREBP-1c in adipose tissue results in a complete loss of adipose tissue, i.e. lipodystrophy, with development of massive fatty liver. Liverspecific overexpression of SREBP-1c results in massively increased amount of visceral adipose tissue and hepatic lipid accumulation. Although both animal models display features of fatty liver the overlap of differentially regulated genes (Mouse 430 2.0, Affymetrix) is rather small indicating distinct mechanisms underlying the development of fatty liver, including genes specific for fatty acid chain elongation or breakdown. To follow this observation we investigated the lipid profile in serum and liver biopsies. Genotype specific lipid profiles are observed thus displaying specific lipid signatures, i.e. the desaturation index is gradually increased in both fatty liver models compared to wildtype mice. This observation goes along with the regulation of SCD gene family.

Conclusion: These mouse models allow the elucidation of the pathogenetic role of the transcription factor SREBP-1c, but also the investigation of different pathophysiological mechanisms of lipid accumulation in liver and their implications for metabolism.

Workshop: FAT DEPOTS, INSULIN RESISTANCE AND ATHEROSCLEROSIS

ROLE OF LIPOPROTEINS IN PANCREATIC BETA-CELL SURVIVAL, PROLIFERATION AND FUNCTION

Rahel Anja Sibler[1], S. Rütti[2], J.A. Ehses[2], R. Prazak[2], D.T. Meier[2], L. Rohrer[1], M.Y. Donath[2], A. von Eckardstein[1]

[1]Institute of Clinical Chemistry, [2]Division of Endocrinology and Diabetes, University Hospital Zurich, Zurich, Switzerland

Diabetic dyslipidemia is characterized by low levels of high density lipoproteins (HDL), hypertriglyceridemia and the presence of small dense low density lipoproteins (LDL). This condition even precedes the manifestation of diabetes mellitus and increases the risk for developing type 2 diabetes mellitus. We therefore investigated the role of these lipoproteins on function, proliferation and survival of human and murine beta-cells. HDL was shown to decrease spontaneous as well as glucose and interleukin-1-beta induced apoptosis in human and murine beta-cells. ApolipoproteinA-I and sphingosine-1-phosphate, two major HDL components, protect islet beta-cells similarly to HDL. LDL on the other hand decreased human and mouse beta-cell proliferation by a mechanism which is independent of the LDL receptor. Furthermore LDL decreased beta-cell function as assed by glucose stimulated insulin secretion by a mechanism which is dependent on the LDL receptor.

Decreasing beta-cell mass and function drive the manifestation of relative insulin deficiency and hence type 2 diabetes mellitus. The effects of HDL and LDL on pancreatic beta-cell survival, proliferation and function point to a causal role of these lipoproteins in the development of type 2 diabetes mellitus. Accordingly they are possible therapeutic targets not only for preventing the clinical sequelae but also the manifestation of type 2 diabetes mellitus.

Workshop: FAT DEPOTS, INSULIN RESISTANCE AND ATHEROSCLEROSIS

NON-ALCOHOLIC FAT LIVER DISEASE IS ASSOCIATED WITH INCREASED VISCERAL FAT AND INSULIN RESISTANCE

Vesna Dimitrijevic-Sreckovic[1], B. Sreckovic[2], P. Djordjevic[1], M. Civcic[1], V. Barac[3], I. Soldatovic[4], M. Stojanovic[1]

[1]Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [2]Bezanijska Kosa Clinical Center, [3]Institute of Medical Biochemistry, [4]Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia

Background: Hepatic fat accumulation in childhood obesity is associated with increased visceral fat and insulin resistance (IR). IR results in fat deposition in the liver and occurrence of non-alcoholic fat liver disease (NAFLD).

Methods: The study included 173 obese individuals aged 7 to 30 classified into 3 groups: I-children (7-15), II-adolescents (16-20) and III-youth (20-30). Three of the following five criteria were used for MS diagnosis: waist circumference >90Pct; triglycerides >1.7mmol/l; HDL-cholesterol< 1.0mmol/l; hypertension>90Pct; glycemia>6.0mmol/L. Patients with less than three afore mentioned criteria were considered patients with pre-MS. OGTT was used to evaluate the extent of disorder. Insulin sensitivity was determined by HOMA IR. SGOT, SGPT and γ-GT were liver function parameters. Liver ultrasonography was used to diagnose NAFLD.

Results: NAFLD, increasing considerably with age, was found in 7.3% children, 18.9% adolescents and 29.0% youth ($p< 0.05$). Logistic regression analysis indicated the most important NAFLD factors: body weight, LDL-cholesterol, uric acid, insulins - 0min,120min, HOMA IR, PAI-l and SGPT. Patients with NAFLD had increased WC (110.7±11.9cm), LDL-cholesterol (3.3±1.0mmol/l), triglycerides (1.81±1.15mmol/l), uric acid (383.8±86.3), insulins 0min (61.1±81.3U/l) and 120min (93.1±108.4U/l), HOMA IR (14.7±4.4±19.3μmol/mU/ml), PAI-1 (7.3±0.6U/ml), SGPT (56.7±20.9U/l), γ-GT (44.1±22.8U/l). Patients without NAFLD had normal SGPT, γ-GT, uric acid and increased WC (98.6±16.7cm), insulins 0min (21.6±31.3U/l) and 120 min (44.3±49.5U/l), HOMA IR (6.2±3.4μmol/mU/ml), triglycerides (1.74±1,63mmo/l), PAI-1 (6.0±1.4U/ml) but lower than NAFLD patients.

Conclusion: NAFDL may be the liver sign of pre-MS and MS children, adolescents and youth associated with visceral obesity, IR, lipid status disturbance, thrombotic and inflammatory factors.

Workshop: FAT DEPOTS, INSULIN RESISTANCE AND ATHEROSCLEROSIS

GENETIC VARIATION WITHIN ADIPONUTRIN IS ASSOCIATED WITH LIPOPROTEIN METABOLISM AND LIVER FUNCTION

Barbara Kollerits[1], S. Coassin[1], N.D. Beckmann[2], A. Teumer[3], S. Kiechl[4], A. Döring[5], M. Kavousi[6], S.C. Hunt[7], C. Lamina[1,5], B. Paulweber[8], Z. Kutalik[2], M. Nauck[9], C.M. van Duijn[6], I.M. Heid[5,10], J. Willeit[4], A. Brandstätter[1], T.D. Adams[7], V. Mooser[11], Y.S. Aulchenko[6], H. Völzke[12], F. Kronenberg[1]

[1]Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria, [2]Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland, [3]Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany, [4]Department of Neurology, Innsbruck Medical University, Innsbruck, Austria, [5]Helmholtz Zentrum München, Neuherberg, Germany, [6]Department of Epidemiology, Erasmus MC, Rotterdam, Rotterdam, The Netherlands, [7]Cardiovascular Genetics Division, University of Utah, School of Medicine, Salt Lake City, UT, USA, [8]First Department of Internal Medicine, Paracelsus Private Medical University Salzburg, Salzburg, Austria, [9]Institute of Clinical Chemistry and Laboratory Medicine, University of Greifswald, Greifswald, [10]Institute of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, Germany, [11]Genetics Division, R&D GlaxoSmithKline, King of Prussia, PA, USA, [12]Institute for Community Medicine, University of Greifswald, Greifswald, Germany

Objective: Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase activity regulated by fasting and feeding. Recent genome-wide association studies identified adiponutrin to be associated with hepatic fat content and liver function, indicating that adiponutrin might be involved in hepatic lipoprotein metabolism. We aimed 1) to elucidate the association of common variants within adiponutrin and parameters of lipoprotein metabolism and 2) with liver function in up to eight independent West-Eurasian study populations including 23,274 individuals.

Methods: This study is based on six population-based studies [Bruneck (n=800), KORA S3/F3 (n=1644), KORA S4/F4 (n=1814), CoLaus (n=5435), SHIP (n=4012), Rotterdam (n=5967)], the SAPHIR Study as a healthy working population (n=1738) and the Utah Obesity Case-Control Study including a group of 1037 severely obese individuals (average BMI 46 kg/m^2) and 827 controls from the same geographical region of Utah.

Results: We observed a strong additive association of a common nonsynonymous variant within adiponutrin (rs738409) with age-, gender-, and alanine-aminotransferase-adjusted lipoprotein concentrations: each copy of the minor allele decreased levels of total cholesterol on average by 2.43 mg/dl (p=0.000001), non-HDL cholesterol levels by 2.35 mg/dl (p=0.000002) and LDL cholesterol levels by 1.48 mg/dl (p=0.0008). Moreover, the rs738409 variant is strongly associated with the liver enzymes ALT and AST, following a recessive model.

Discussion: In conclusion, our study suggests that adiponutrin is related to processes of lipid and energy metabolism with a special focus on apolipoprotein B-containing lipoproteins, as well as hepatic dysfunction.

Workshop: NOVEL ANTI-ATHEROSCLEROTIC TREATMENT STRATEGIES

RVX-208 GIVEN ORALLY RAISES PLASMA APOA-I AND HDL IN HUMAN CLINICAL TRIALS

A. Gordon[1], J. Johansson[1], G. Wagner[1], F. Chiacchia[2], **Norman Wong**[2]

[1]Resverlogix Corporation, San Francisco, CA, USA, [2]Resverlogix Corporation, Calgary, AB, Canada

Introduction: RVX-208 a novel small molecule that acts orally is known to increase apoA-I production in vitro and in vivo.

Objective: To study RVX-208 in humans.

Methods: Standard clinical trials procedures.

Results: Both phase 1a and 1b/2a human trials data totaling >160 subjects showed RVX-208 to be safe and well tolerated. In the 1a study, 18 subjects received 2-8 mg/kg/d of RVX-208 orally for 7 days (d) vs. 6 placebos, the apoA-I, prebeta1- HDL and ABCA1 mediated cholesterol efflux rose by 10, 42 and 10%, respectively ($p < 0.05$) with trending towards higher HDL-c (10%) and alpha1-HDL (21%). In the 1a/2b study, 18 subjects received 2 mg/kg/d (low), another 18 subjects took 6 mg/kg/d (high) vs. 12 placebos for 28d. The apoA-I rose significantly in low- and high-dosed subjects to 5.1 and 8.2% within 8d, respectively. At 28d there was a further increase to 6.5 and 10.4%, respectively vs. placebo. Similarly, HDL-c in low and high-dosed subjects was higher by 6.2% and 9.2 % at 8d, respectively. At 28d HDL-c rose further to 7.4 and 15.1%, respectively vs. placebo. The apoA-I and HDL-c increases were more robust in low HDL subjects. 2D-PAGGE analysis of sera from treated subjects showed a striking 43% (57% in low HDL subjects) rise in large alpha1- HDL particles at 28d.

Conclusions: RVX-208 raises endogenous apoA-I production and plasma; apoA-I, HDL-c, and especially alpha1- HDL, in a dose and time dependent manner. These changes should enhance reverse cholesterol transport and thus regress atherosclerosis to reduce CVD events.

Workshop: NOVEL ANTI-ATHEROSCLEROTIC TREATMENT STRATEGIES

INHIBITION OF HEPATIC SR-BI BY RNA INTERFERENCE PROTECTS FROM ATHEROSCLEROSIS IN RABBITS

Egon Demetz, I. Tancevski, U. Stanzl, K. Duwensee, W. Schgoer, A. Ritsch, C. Heim, E. Huber, J.R. Patsch, P. Eller

Medical University Innsbruck, Innsbruck, Austria

Background: Scavenger receptor B type I (SR-BI) plays a key role in high-density lipoprotein metabolism, whole body cholesterol homeostasis and reverse cholesterol transport. Therefore, we investigated the influence of SR-BI inhibition on lipoprotein metabolism in an animal expressing cholesteryl ester transfer protein.

Methods and results: In the current study we used RNA interference to inhibit expression of SR-BI. We designed a small hairpin RNA plasmid specific for SR-BI, pENTR/rSR-BI/214, and validated it *in vitro* in HUH7 cells stably transfected with SR-BI. In order to target hepatic tissue of New Zealand White rabbits *in vivo*, pENTR/rSR-BI/214 was complexed with galactosylated poly-lysine allowing an organ-selective receptor-mediated gene transfer. 10 days after intravenous injection, fast protein liquid chromatography of pooled plasma was performed. Animals injected with pENTR/rSR-BI/214 (30 µg/kg) showed an elevation in low-density lipoprotein cholesterol of 40% when compared to rabbits treated with a scrambled control sequence. Furthermore, weekly injection of pENTR/rSR-BI/214 resulted in a 40% reduction of atherosclerotic lesions within the aortic arch after 8 weeks ($P < 0.001$).

Conclusions: We conclude that SR-BI inhibition in liver of rabbits resulted in a faster turnover of high-density lipoproteins due to elevated cholesteryl ester transfer to larger lipoproteins. The observed shift in lipoproteins with an increase in low-density lipoproteins turned out to be atheroprotective in the presented animal model and, thus, warrants further research of this new pharmacological principle.

Workshop: NOVEL ANTI-ATHEROSCLEROTIC TREATMENT STRATEGIES

THE CXCR7 LIGAND CCX771 REDUCES NEOINTIMA FORMATION AFTER VASCULAR INJURY AND ATHEROSCLEROSIS IN APOE$^{-/-}$ MICE

Xiaofeng Li[1], M. Penfold[2], T.J. Schall[2], C. Weber[1], A. Schober[1]

[1]Institute for Molecular Cardiovascular Research, RWTH Aachen, Aachen, Germany, [2]ChemoCentryx, Inc., Mountain View, CA, USA

The chemokine receptor CXCR7 plays a role in cell survival, adhesion, tumor growth and development by binding to CXCL12 (SDF-1a) and CXCL11 (I-TAC). CCX771 is a highly specific CXCR7 ligand, which has been shown to affect SDF-1a/ITAC binding to CXCR7 and modulate b-arrestin coupling. We studied the role of CCX771 in atherosclerotic vascular disease, such as neointima formation after vascular injury and diet-induced atherosclerosis.Wire-induced injury of the carotid artery was performed in ApoE$^{-/-}$ mice on western-type diet. Mostly endothelial CXCR7 immunostaining was evident at 1 week after injury. Mice were treated with CCX771 (10mg/kg/d, s.c., n=8) or the solvent Captisol (10%, n=8) for 28 days. Plasma CCX771 levels were sufficiently high to provide full receptor coverage at trough. CCX771 reduced neointima formation by 35% due to a diminished neointimal macrophage content. SMC and T-cell content, however, were not affected by CCX771. CXCL12-mediated mobilization of Sca-1^{+}/Lin^{-} progenitor cells 24h after injury was similar in CCX771- and Captisol-treated mice.In the atherosclerosis model, ApoE-/- mice on high-fat diet were treated with CCX771 (10mg/kg/d, s.c., n=10) or Captisol (10%, n=10) for 3 months. Analysis of the en face prepared throcaoabdominal aorta after Oil-Red-O staining demonstrated a 41% decrease of aortic lipid deposition in CCX771-treated mice ($P < 0.05$). Determination of body weight, serum AST, ALT, creatinine, and C-reactive protein revealed no differences between CCX771- and Captisol-treated mice in the wire-injury and atherosclerosis model.We demonstrate a protective role of the CXCR7 ligand CCX771 in atherosclerotic vascular disease through reduced macrophage recruitment.

Workshop: REGENERATING CELLS IN THE CARDIOVASCULAR SYSTEM

PROTECTION AGAINST ATHEROSCLEROSIS IN CAV-1$^{-/-}$ MICE IS DUE TO CAV-1$^{-/-}$ IN NON-HEMATOPOIETIC CELLS

David Engel[1], L. Beckers[1], E. Wijnands[1], M.J.A.P. Daemen[1], R. Stan[2], E.A.L. Biessen[1], E. Lutgens[1]

[1]Pathology, Maastricht University Medical Center, Maastricht, The Netherlands, [2]Pathology, Dartmouth Medical School, Hanover, NH, USA

Caveolin-1 is a an integral plasma membrane protein and has been shown to play a crucial role in cholesterol homeostasis and activation of T lymphocytes. As these processes play a role in atherosclerosis we investigated the inflammatory and immunological potential of Cav-1 in atherosclerosis.

We compared ApoE$^{-/-}$/Cav-1$^{+/+}$ with ApoE$^{-/-}$/Cav-1$^{-/-}$ mice to assess information on atherosclerosis by looking at plaque size, number and phenotype. Additionally we performed a bone marrow transplantation to investigate the effect of hematopoietic and non-hematopoietic Cav-1 deficiency. T cell subsets were examined by FACS analysis.

ApoE$^{-/-}$Cav1$^{-/-}$ mice presented with a 6.9 to 15 fold reduction in total plaque area ($p< 0.05$). The plaques of the Cav-1$^{-/-}$ mice showed a 43% reduction in MΦ and a 40% reduction in CD3^{+} T-cell content. FACS analysis revealed that ApoE$^{-/-}$/Cav-1$^{-/-}$ mice had more CD3^{+} T-cells (+17%, $p< 0.05$), relatively more CD8^{+} cytotoxic T-cells (+6%, $p< 0.05$) and regulatory T-cells (+37%, $p< 0.01$) but less CD4^{+} helper T-cells (-4%, $p< 0.05$) than their ApoE$^{-/-}$/Cav-1$^{+/+}$ counterparts.

The BMT revealed that Cav-1$^{+/+}$ mice receiving either Cav-1$^{+/+}$ or Cav-1$^{-/-}$ bone marrow had larger ($p< 0.01$, 5.1 to 4.5 fold) and more plaques ($p< 0.05$, 2 to 1.6 fold) compared to the Cav-1$^{-/-}$ mice receiving the same bone marrow. The Cav-1$^{-/-}$ mice which received either Cav-1$^{+/+}$ or Cav-1$^{-/-}$ bone marrow had less CD8^{+} and regulatory T-cells ($p< 0.05$, 16%, 20%) but more CD4^{+} T-cells ($p< 0.05$, 5 to 8 %).

Our results suggest a pivotal role for the non-hematopoietic Cav-1$^{+/+}$ cells in plaque initiation and progression.

Workshop: REGENERATING CELLS IN THE CARDIOVASCULAR SYSTEM

UROKINASE RECEPTOR MEDIATES MOBILIZATION, MIGRATION AND DIFFERENTIATION OF MESENCHYMAL STEM CELLS

Krishna Vallabhaneni[1], S. Tkachuk[1], J. Kiyan[1], N. Shushakova[2], I. Dumler[1], G. Eden[1]

[1]Department of Nephrology, Medizinische Hochschule Hannover, [2]Phenos GmbH, Hannover, Germany

Multipotent mesenchymal stem cells (MSCs) have regenerative properties and are recognized as putative players in the pathogenesis of arterial remodeling, plaque stability and angiogenesis.The underlying molecular mechanisms remain, however, sparsely explored. Our study was designed to elucidate a probable role for the multifunctional urokinase (uPA)/urokinase receptor (uPAR) system in MSCs regulation. Though uPAR has been implicated in a broad spectrum of cardiovascular pathophysiological processes, nothing is known about uPAR in MSCs.

We provide evidence that uPAR was required to mediate MSCs mobilization from the bone marrow of mice stimulated with G-CSF *in vivo*. Insignificant amount of MSCs were mobilized in response to G-CSF in uPAR-deficient mice, whereas in wild type animals G-CSF treatment resulted in 11-fold increase of mobilized MSCs. Experiments on MSCs migration using *in vitro* injury assay demonstrated uPAR involvement in this process. uPAR downregulation by means of RNA silencing lead to inhibition of MSCs migration. We established conditions for MSCs differentiation into vascular smooth muscle cells (VSMCs) that was monitored by changes in cell morphology and expression of specific marker proteins. MSCs differentiation into VSMCs positively correlated with uPAR expression level. uPAR down- or upregulation by means of lentiviral transgene expression resulted in inhibition or stimulation of MSCs differentiation correspondingly. These data suggest a multifaceted function of uPAR in MSCs biology contributing to vascular repair. uPAR might guide and control the trafficking of MSCs to the vascular wall in response to injury or ischemia and their differentiation towards functional VSMCs at the site of arterial injury.

Workshop: REGENERATING CELLS IN THE CARDIOVASCULAR SYSTEM

HIGH-DENSITY-LIPOPROTEIN CHOLESTEROL IS A STRONG DETERMINANT OF ENDOTHELIAL PROGENITOR CELLS NUMBER AND FUNCTION IN HYPERCHOLESTEROLEMIC SUBJECTS

Fabio Rossi, C. Bertone, F. Montanile, F. Miglietta, C. Lubrano, V. Santiemma

Sapienza University of Rome, Roma, Italy

Endothelial progenitor cells (EPC) can repair the endothelial layer and are considered a component of the cardiovascular system. EPC number and function may change under pathological conditions, including cardiovascular risk factors. The study was carried out to investigate circulating EPC number, in vitro function and relationship with LDL-C, HDL-C and endothelium-dependent vasodilatation in hypercholesterolemic subjects.

41 male and 39 female subjects, at their first diagnosis of hypercholerolemia were consecutively recruited in Outpatient Service of the Medical Pathophysiology Department of Rome Sapienza University. Inclusion criteria: Age >35 and < 45; LDL-cholesterol plasma level >130mg/dl with normal (≥50mg/dl females and ≥40mg/dl males) or low HDL-C; absence of any concomitant disorders and/or drug treatment. In high LDL-C patients, circulating EPC number was decreased versus a control group matched for age and sex. EPC capability to migrate was impaired as well. This pattern was far less evident in the normal HDL-C subgroup.

The endothelium-dependent vasodilatation (EDV) was significantly decreased according to the HDL-C in male but not in female subjects. Univariate analysis showed a direct correlation between EPC number and endothelium-dependent vasodilatation. The association between EPC number and EDV persisted after adjustment for sex, age and HDL-C, which were all significantly correlated to EDV, suggesting a protective role of EPC on endothelium in vivo.

Our study documented that in hypercholesterolemic subjects, HDL-C is a strong determinant of EPC number and function, and EPC number decrease is an independent risk factor for endothelial dysfunction.

Workshop: REGENERATING CELLS IN THE CARDIOVASCULAR SYSTEM

RATIO OF MICROPARTICLES TO ENDOTHELIAL PROGENITOR CELLS, A MARKER OF VASCULAR DYSFUNCTION INDUCED BY COMBINED HYPERTENSION AND HYPERCHOLESTEROLEMIA; IRBERSARTAN EFFECT

Adriana Georgescu[1], N. Alexandru[1], D. Popov[1], E. Andrei[1], I. Titorencu[1], E. Dragan[1], C. Tarziu[2], S. Ghiorghe[2], E. Badila[2], D. Bartos[2], M. Simionescu[1]

[1]Institute of Cellular Biology and Pathology 'Nicolae Simionescu', [2]Emergency Hospital, Bucharest, Romania

This study aimed to

(a) employ our newly designed model, the hypertensive-hypercholesterolemic hamster (HH) to find whether a correlation exist between circulating microparticles (MPs), endothelial progenitor cells (EPCs) and their contribution to vascular dysfunction and

(b) to assess the effect of irbersartan treatment on HH animals (HHI). Aortic arch and mesenteric resistance arteries were explanted from HH, HHI and control (C) hamsters. The results showed that compared to C group, HH displayed:

(i) a significantly increase in plasma cholesterol and triglyceride concentration, and an enhanced systolic and diastolic arterial blood pressure, and heart rate;

(ii) a marked elevation of MPs and a significant decrease in EPCs;

(iii) structural modifications of arterial wall correlated with increased protein expression of MMP2, MMP9, MMP12, TIMP1, TIMP2 and collagen type I and III;

(iv) a considerably altered reactivity of the arterial wall closely correlated with MPs and EPCs adherence; (v) an inflammatory process characterised by augmented expression of P-Se

lectin, E-Selectin, vWF, TF, IL-6, MCP-1, RANTES, eNOS, VEGF and SDF-1.

Additionally, the experiments showed the potential of irbersartan to correct all altered parameters in HH. In conclusion, hypercholesterolemia associated with hypertension is accompanied by structural modifications and expression of pro-inflammatory molecules by the vessel wall, alteration of the vascular tone, enhanced release of MPs and reduced EPCs; the ratio between the latter two may be considered a marker of vascular dysfunction. Irbersartan that exhibit a pharmacological control on the levels of MPs and EPCs has the potential to restore homeostasis of the arterial wall.

Workshop: GENETICS IN ATHEROSCLEROSIS

TELOMERES ARE SHORTER IN PATIENTS WITH POLYGENIC AND MONOGENIC FORMS OF CORONARY HEART DISEASE

C.G. Maubaret[1], **Klelia D. Salpea**[1], A. Jain[2], J.A. Cooper[1], A. Hamsten[3], J. Sanders[4], H. Montgomery[5], A. Neil[6], D. Nair[2], S.E. Humphries[1], HIFMECH Consortium; Simon Broome Research Group

[1]Department of Medicine, University College London, [2]Department of Clinical Chemistry, Royal Free Hospital, London, UK, [3]Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska University Hospital, Stockholm, Sweden, [4]Department of Epidemiology and Public Health, [5]Institute for Human Health and Performance, University College London, London, [6]Division Public Health & Primary Health Care, Univesrsity of Oxford, Oxford, UK

Introduction: Leukocyte telomere length (LTL) has been previously associated with coronary heart disease (CHD). The aim of our study was to confirm this association in cases of different CHD etiology; the common polygenic form and, for the first time, with CHD caused by familial hypercholesterolaemia (FH).

Methods: Two CHD case-control studies, consisted of 598 white male patients who survived a first myocardial infarction (MI) (< 60 years) recruited from four European countries and 413 coronary artery bypass graft (CABG) patients recruited in the UK compared to 653 region matched controls, were used. Additionally, two groups of 367 and 94 FH patients of whom 145 and 17 respectively had premature CHD were recruited from the UK. Leukocyte telomere length (LTL) was measured using a quantitative PCR-based method.

Results: Age-adjusted LTL was significantly shorter in premature MI cases (7.85 kb, SD 4.01) compared to controls (8.04 kb, SD 4.46) (p=0.04) as well as in CABG cases (6.89 kb, SD 4.14) compared to controls (7.53 kb, SD 5.29) (p=0.007). In the combined sample of the two FH studies, age-adjusted LTL was shorter in the patients with CHD (8.68 kb, SD 4.65) compared to those without (9.23 kb, SD 4.83) (p=0.012). Apart from a consistent negative correlation with age, no other CHD risk factor was associated with LTL across the studies.

Conclusion: The present data confirms the shortened telomeres as a marker of CHD, possibly reflecting the ageing of vascular wall, and extends this association to those with monogenic and polygenic forms of CHD.

Workshop: GENETICS IN ATHEROSCLEROSIS

INCREASED *ANRIL* EXPRESSION IS ASSOCIATED WITH ATHEROSCLEROSIS SEVERITY AT CHROMOSOME 9P21

Lesca Holdt[1], F. Beutner[1], M. Scholz[2], S. Gielen[3], G. Gäbel[4], H. Bergert[4], G. Schuler[3], J. Thiery[1], D. Teupser[1]

[1]Institute of Laboratory Medicine, [2]Institute of Medical Informatics, Statistics and Epidemiology (IMISE), [3]University Leipzig - Heart Center, Department of Internal Medicine/Cardiology, University Leipzig, Leipzig, [4]Department of General, Thoracic and Vascular Surgery, University of Dresden, Dresden, Germany

Objectives: Genome-wide association studies have revealed a locus of coronary artery disease (CAD) susceptibility on human chromosome (Chr) 9p21. No known protein-coding genes reside within the identified haplotype block except for antisense non-coding RNA in the INK4 locus (ANRIL). We thus investigated whether transcript expression +/- 200kb of the core haplotype block was affected by the genotype at this locus and associated with atherosclerosis risk.

Methods and results: We replicated the locus for CAD (P=0.007, OR=1.28) and other manifestations of atherosclerosis such as carotid plaque (P=0.003, OR=1.31) in the Leipzig Heart Study, a cohort of 1134 patients with varying degree of angiographically assessed CAD. Expression analysis in peripheral blood mononuclear cells (n=1098) revealed that transcripts EU741058 and NR_003529 of antisense non-coding RNA in the INK4 locus (ANRIL) were significantly increased in carriers of the risk haplotype ($P=2.1x10^{-12}$ and $P=1.6x10^{-5}$, respectively). In contrast, transcript DQ485454 remained unaffected, suggesting differential expression of ANRIL transcripts at Chr9p21. Results were replicated in whole blood (n=769) and atherosclerotic plaque tissue (n=41). Moreover, expression of ANRIL transcripts was directly correlated with severity of atherosclerosis (EU741058 and NR_003529, P=0.02 and P=0.001, respectively). No consistent association of Chr9p21 or atherosclerosis was found with expression of other genes such as CDKN2A, CDKN2B, C9orf53 and MTAP.

Conclusion: Our data provide robust evidence for an association of ANRIL with atherosclerosis at the Chr9p21 locus in a large cohort. Further studies are needed to unravel the molecular mechanism through which ANRIL influences atherosclerosis risk.

Workshop: GENETICS IN ATHEROSCLEROSIS

COMMON GENETIC VARIANTS ASSOCIATED WITH LOW LP(A) KRINGLE-IV COPY NUMBER, HIGH LP(A) CONCENTRATION, AND INCREASED RISK OF CORONARY HEART DISEASE

Udo Seedorf[1], J. Peden[2], J. Hopewell[2], T. Kyriakou[2], A. Goel[2], S. Heath[3], S. Parish[2], S. Barlera[4], M. Grazia[4], S. Rust[1], D. Bennett[2], A. Silveira[5], A. Malarstig[5], F. Green[6], M. Lathrop[3], B. Gigante[5], K. Leander[5], U. de Faire[5], R. Clarke[2], A. Hamsten[5], R. Collins[2], H. Watkins[2], PROCARDIS Consortium

[1]Leibniz-Institut für Arteriosklerosеforschung, Münster, Germany, [2]University of Oxford, Oxford, UK, [3]Centre Nationale de Genotypage, Paris, France, [4]Mario Negri Institute, Milan, Italy, [5]Karolinska Institutet, Stockholm, Sweden, [6]University of Surrey, Guildford, UK

Background: Lp(a) lipoprotein is an emerging risk factor for coronary artery disease, however, the genetic determinants of Lp(a) and their relevance for coronary disease risk are incompletely understood.

Methods: We used a novel gene chip containing 48,742 single-nucleotide polymorphisms in 2100 CVD candidate genes to test for association in 3145 coronary disease cases and 3352 controls. Significant associations were validated in 3 independent populations involving 4846 additional coronary disease cases and 4594 controls.

Results: Three chromosomal regions (6q26-27, 9p21, and 1p13) were strongly associated with coronary disease risk. The LPA locus on 6q26-27 encoding Lp(a) showed the most significant effect. We identified a common variant (rs10455872) at the LPA locus with an odds ratio for coronary disease of 1.70 (95%CI 1.49-1.95), and another independent variant (rs3798220) with an odds ratio of 1.92 (95%CI 1.48-2.49). Both variants were strongly associated with high Lp(a) concentration, low kringle-IV copy number, and small Lp(a) size. Replication studies confirmed the effects of both variants on Lp(a) concentrations and coronary disease risk. In a meta-analysis, a LPA genotype score involving both SNPs had odds ratios for coronary disease of 1.51 (95%CI 1.38-1.66) for one and 2.57 (95%CI 1.80-3.67) for two or more variants. After adjustment for Lp(a) concentration, the association of LPA genotype score with coronary disease risk was abolished.

Conclusions: Two LPA variants are strongly associated higher Lp(a) concentrations, lower Lp(a) kringle-IV copy number and higher coronary disease risk.

Workshop: GENETICS IN ATHEROSCLEROSIS

RISK PREDICTION OF MYOCARDIAL INFARCTION USING A WEIGHTED GENETIC SCORE--THE GERMAN MI FAMILY STUDY

Patrick Linsel-Nitschke[1], C. Willenborg[1], A. Schaefer[2], S. Schreiber[2], C. Hengstenberg[3], H. Schunkert[1], J. Erdmann[1]

[1]Medizinische Klinik II, University of Luebeck, Luebeck, [2]Clinical Molecular Biology, Christian Albrechts University, Kiel, [3]Medizinische Klinik II, University of Regensburg, Regensburg, Germany

Introduction: Recent genome wide association studies discovered several susceptibility genes for myocardial infarction (MI). Individually, these genetic variants confer only a modest risk increase. The goal of the present study was to determine if a score build on the 10 currently known risk alleles with genome-wide significance for MI leads to better risk prediction and discrimination of cases and controls.

Methods: We constructed an additive genetic score using single-nucleotide polymorphisms (SNPs) from 10 MI susceptibility genes, weighting each SNP with its reported effect. We tested by regression analysis ?? this score in three independent case-control samples from the German MI Family study comprising a total number of 3435 controls and 4488 cases.

Results: In each of the three case-control cohorts the weighted score derived from 10 MI risk genes was significantly associated with MI risk with odds ratios strongly exceeding the risk conferred by individual SNPs (GerMIFs I OR 3.9 [2.8-5.4], p = 6.23E-16; GerMIFs II OR 3.7 [2.6-5.1], p =4.42E-15; GerMIFs III (KORA) OR 2.4 [1.8-3.2], p =2.77E-09).

Conclusions/interpretation: In our three case-control samples the combination of risk alleles from 10 recently identified MI loci shows a strong additive effect that was consistently replicated. However, on an individual level the discrimination of cases and controls through the genotype score remains difficult. In the near future the addition of further newly identified MI risk loci into the score could improve individual risk prediction and might eventually lead to clinical application of a genetic risk score for MI.

Late Breaking Session

TIE1 ATTENUATION RESULTS IN A LOCATION-SPECIFIC AND SHEAR STRESS-DEFINED REDUCTION IN ATHEROSCLEROSIS

Kel Vin Woo, X. Qu, V. Babaev, M. Linton, R. Guzman, S. Fazio, H.S. Baldwin

Vanderbilt University Medical Center, Nashville, TN, USA

Although it is known that the response of endothelial cells to atherogenic disturbed flow is distinct from that elicited by non-atherogenic laminar flow, the mechanisms involved are poorly understood. Tie1 is an endothelial specific, orphan receptor tyrosine kinase and ablation of Tie1 expression results in embryonic lethality, thus defining a role for Tie1 signaling in atherosclerosis has been elusive.

Surgical insertion of shear stress-modifying casts in mice elicited a specific and rapid attenuation of Tie1 by laminar flow with high shear stress. We also observed that expression of Tie1 was evident only at regions of atherogenic flow. Hence, we hypothesized that Tie1 plays a role in the endothelial response to atherogenic shear stress. We documented a 38% decrease in atherosclerosis in Tie1 heterozygous, apolipoprotein E (ApoE) knockout mice. To further attenuate Tie1 expression *in vivo* and to circumvent embryonic lethality, we developed a loxP mediated, conditional Tie1 allele. We found that an 81% reduction in Tie1 message was associated with a 70% decrease in atherosclerotic lesions, in Tie1-flox, SCL-ER^T-Cre, ApoE null mice compared to Tie1 wild-type mice following tamoxifen-induced gene deletion.

To test our hypothesis *in vitro*, we isolated primary aortic endothelial cells from Tie1-flox:SCL-ERT-Cre immortomice and found that atheroprotective laminar flow decreased Tie1 expression. Tamoxifen-induced Tie1 deletion elevated laminar flow-mediated eNOS expression, and decreased p50 nuclear translocation in response to non-laminar flow. In summary, we found that shear stress conditions that modulate atherogenic events also regulate Tie1 expression and Tie1 may play a novel pro-inflammatory role in atherosclerosis.

Late Breaking Session

VISUALISING INFLAMED ATHEROSCLEROTIC PLAQUES: MOLECULAR IMAGING USING MRI AND TARGETED ULTRASMALL SUPERPARAMAGNETIC PARTICLES OF IRON OXIDE (USPIO)

Joyce Chan[1], C. Monaco[2], K. Bhakoo[3], R. Gibbs[4]

[1]Vascular Surgery, St Mary's Hospital, Imperial College Healthcare NHS Trust, [2]Cytokine Biology of Atherosclerosis, Kennedy Institute of Rheumatology, Imperial College, Charing Cross Campus, [3]Stem Cell Imaging Group, Imperial College, Hammersmith Campus, [4]Vascular Surgery Unit, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, UK

Background and Aims: Inflammation drives atherosclerotic plaque instability and acute thromboembolic events, such as stroke. There is currently no clinical imaging technique available to assess the degree of inflammation associated with plaques. This study aims at visualising and characterising atherosclerosis using targeted USPIO as an MRI probe for detecting inflamed endothelial cells and inflamed atherosclerotic plaques.

Method: The *in vitro* study consists of detection and characterisation of inflammatory markers on activated endothelial cells by immunocytochemistry and anti-E-selectin antibody conjugated USPIO. The *ex vivo* stage involves characterisation of inflammatory markers on human atherosclerotic plaques.

Results: We have established an *in vitro* cellular model of endothelial inflammation induced with TNF-alpha. We have confirmed the inflammation of endothelial cells with both immunocytochemistry and MRI. We are also able to image the inflammation of human atherosclerotic plaques by *ex vivo* MRI.

Conclusion: We successfully developed an *in vitro* model to detect and characterise inflamed endothelial cells by immunocytochemistry and MRI. We are also able to image the inflammation of human atherosclerotic plaques by *ex vivo* MRI. This novel work will allow us to develop agents and protocols for imaging vascular inflammation in atherosclerosis. This provides a new biologically based imaging modality beyond anatomy to identify the 'at risk' group with unstable plaque disease, affording the opportunity for early stroke prevention treatment.

Late Breaking Session

THE GUIDANCE CUE NETRIN-1 PROMOTES ATHEROSCLEROSIS

Janine M van Gils[1], M.C. Stewart[2], K.J. Rayner[1], L.R. Fernandes[3], T.O. McDonald[4], K.D. O'Brien[4], K.J. Moore[1]

[1]NYU Langone Medical Center, New York, NY, [2]Massachusetts General Hospital, Boston, MA, USA, [3]Federal University of Minas Gerais, Belo Horizonte, Brazil, [4]University of Washington, Seattle, WA, USA

The chronic inflammation underlying atherosclerosis is fueled by the persistence of lipid-laden macrophages in the artery wall. However, the mechanisms by which these immune cells become trapped are not well understood. Emerging data suggest that axonal guidance cues typically expressed during development, such as Netrin-1, have additional guidance roles outside the central nervous system in the induction and inhibition of cell migration.

Aim: We hypothesize that Netrin-1 is expressed in atheroma and quenches macrophage emigration from the intima.

Methods and Results: Immunohistochemical analysis of human atherosclerotic lesions revealed abundant expression of Netrin-1 by macrophage foam cells. Incubation of macrophages with oxLDL *in vitro* caused a 3-fold upregulation of Netrin-1 protein. Migration assays showed that recombinant Netrin-1 blocks monocyte migration, via the receptor Unc5b. Likewise, supernatants from oxLDL-stimulated macrophages inhibit monocyte migration, and this effect is reversed by treatment with anti-Unc5b antibodies. These data suggest that lesional expression of Netrin-1 may promote atherosclerosis by retaining macrophages in the intima. To test this, we reconstituted the bone marrow of *Ldlr*$^{-/-}$ mice with *Ntn1*$^{+/+}$ or *Ntn1*$^{-/-}$ hematopoietic stem cells and measured atherosclerosis after 12 weeks of western diet feeding. Chimeric *Ntn1*$^{-/-}$*Ldlr*$^{-/-}$ mice show 40-50% decreases in atherosclerotic lesion area in both the aortic sinus and the aorta enface.

Conclusion: Netrin-1, a negative regulator of macrophage migration, is expressed in the vessel wall and contributes to lesion development. These studies provide insight into the signals that promote macrophage retention in atherosclerosis, and may have wide ranging implications for the understanding of chronic inflammatory disorders.

Late Breaking Session

BASELINE CHARACTERISTICS OF PATIENTS IN THE SATURN STUDY, A COMPARISON OF ROSUVASTATIN VERSUS ATORVASTATIN ON CORONARY ATHEROSCLEROTIC DISEASE BURDEN

PJ Barter[1], J. Chapman[2], C. Ballantyne[3], R. Erbel[4], P. Libby[5], S. Nicholls[6], J. Raichlen[7], V. Cain[7], S. Nissen[6]

[1]Heart Research Institute, Sydney, NSW, Australia, [2]Hôpital de la Pitié, Paris, France, [3]Baylor College of Medicine, Houston, TX, USA, [4]West German Heart Center, Essen, Germany, [5]Brigham & Women's Hospital, Boston, MA, [6]Cleveland Clinic Foundation, Cardiovascular Medicine, Cleveland, OH, [7]AstraZeneca, Wilmington, DE, USA

Background: Intravascular ultrasound (IVUS) can assess effects of interventions on coronary atherosclerotic burden. SATURN (Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin versus Atorvastatin; NCT00620542) compares the effect of rosuvastatin versus atorvastatin on coronary artery disease (CAD) progression.

Methods: This multicentre, double-blind study, initially randomized adult patients to rosuvastatin 20 mg or atorvastatin 40 mg/day for 2 weeks. Patients with LDL-C < 3.0 mmol/L and triglyceride level < 5.65 mmol/L were re-randomized to receive treatment with rosuvastatin 40 mg or atorvastatin 80 mg for 2 years. The primary endpoint is IVUS-assessed change in the percent atheroma volume in a ≥40 mm segment of a single coronary artery.The study will also evaluate lipid levels and inflammatory biomarkers.

Results: 1385 patients were randomized into the treatment period (mean age, 57.6 years; 72% male). By BMI, 45% of patients were overweight and 38% were obese. At least one risk factor was present in 91.8%, including hypertension (68.2%), low HDL-C (39.2%), family history of premature CAD (38.6%) and smoking in the last month (31.9%). Baseline LDL-C, HDL-C, total cholesterol and triglyceride values were 3.1, 1.2, 5.0 and 1.6 mmol/L, respectively, and mean ApoB/ApoA-1 ratio was 0.86. Current treatments included aspirin (86.4% patients), beta-blockers (72.5%), statins (61.7%), ACE inhibitors (49.1%), and ARBs (14.4%). 23.8% had previous myocardial infarction and 21.9% had undergone percutaneous coronary interventions.

Conclusions: SATURN results will allow comparison of the efficacy of high-dose statins in a representative population of CAD patients with modifiable risk factors.

Late Breaking Session

PHASE 3 STUDY OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITOR (MTP-I) LOMITAPIDE IN SUBJECTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HOFH): 56-WEEK RESULTS

Marina Cuchel[1], E. Meagher[1], A.D. Marais[2], D.J. Blom[2], H. du Toit Theron[3], P.K. Shah[4], R.A. Hegele[5], L.T. Bloeden[6], W.J. Sasiela[6], D.J. Rader[1]

[1]Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA, USA, [2]University of Cape Town, Cape Town, [3]Netcare Private Hospital, Bloemfontein, South Africa, [4]Division of Cardiology and Atherosclerosis Research Center, Cedars-Sinai Heart Institute, Los Angeles, CA, USA, [5]Vascular Biology Research Group, Robarts Research Institute and Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada, [6]Aegerion Pharmaceuticals, Bridgewater, NJ, USA

Objectives: To assess efficacy and safety of the MTP-I lomitapide in adults with HoFH. We present available data on 11 subjects treated for 56 weeks.

Methods: During the 26-week titration period, lomitapide was uptitrated from 5 mg to maximum tolerated dose, and added to current, stable lipid-altering treatments. Background therapies (e.g. LDL apheresis) could be modified after week 26.

Results: Mean dose of lomitapide was 42 mg at 26 weeks and 38 mg at 56 weeks (range=20−60 mg for each). Mean (±SD) LDL-C levels declined from baseline by 43±35% at 26 weeks and 38±26% at 56 weeks. At 56 weeks, 6 (55%) of the 11 subjects had LDL-C < 150 mg/dL. HDL-C declined during the titration period (−16±15%) but rebounded by week 56 (+1±33%). Body weight decreased by 4.4±4.1% at week 26 and 3.8±5.2% at week 56. Lomitapide was well tolerated, with 3 subjects (of 29 total enrolled) discontinuing, all because of gastrointestinal effects. Transaminase elevations (5.00−9.99XULN) were observed in 2 subjects (of 11 completing 56 weeks) during titration, requiring temporary dose reductions. No elevations >5XULN were observed beyond week 26. Lomitapide did not affect bilirubin, vitamin A, or vitamin E status. Hepatic fat increased from a baseline mean (±SD) of 1.15±1.5% to 7.6±6.6% at week 26, then declined to 3.9±4.3% at week 56.

Conclusions: In a preliminary analysis of 11 adults with HoFH treated with lomitapide, mean LDL-C reduction was 38% at 56 weeks. Hepatic fat declined significantly between weeks 26 and 56, approaching baseline levels despite continued treatment.

Late Breaking Session

EFFICACY OF 15 YEARS OF GENETIC CASCADE SCREENING FOR FAMILIAL HYPERCHOLESTEROLEMIA IN THE NETHERLANDS IN PREVENTION OF CORONARY ARTERY DISEASE

Roeland Huijgen[1], J. Versmissen[2], D.M. Oosterveer[2], I. Kindt[3], E.J.G. Sijbrands[2], J.J.P. Kastelein[1]

[1]Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, [2]Dept of Cardiovascular Genetics & Dept of Pharmacology, Vascular and Metabolic Diseases, Erasmus Medical Center, Rotterdam, [3]Foundation for Identification of Persons with Inherited Hypercholesterolemia (StOEH), Amsterdam, The Netherlands

Objective: Familial hypercholesterolemia (FH) is associated with a severely increased risk of coronary artery disease (CAD). Genetic screening for FH is ongoing in the Netherlands since 1994 and after such diagnosis 85% used cholesterol-lowering medication (Huijgen *PLoS ONE* 2010). Statin treatment reduces the risk of a first CAD event by 80% in clinically diagnosed FH patients (Versmissen *BMJ* 2008). If CAD risk attributable to FH is similar in clinically and genetically diagnosed patients, one could predict the treatment-induced risk reduction after genetic diagnosis.

Methods: We compared CAD event free survival in 1,338 patients clinically diagnosed with FH and 11,136 relatives identified with FH through genetic screening to that of 20,057 screened relatives without FH before 1990 (pre-statin era). We used a Cox-proportional hazard model, adjusted for traditional cardiovascular risk factors. We estimated the number of CAD events after genetic FH diagnosis until expected age of death.

Results: The risk of CAD was significantly increased, in both clinically and genetically diagnosed FH patients, compared to unaffected relatives, peaking at the age of 35 (RR 11.9; 95%CI 5.2 to 27.1 and RR 11.8; 95%CI 6.4 to 21.8, respectively). In 6,366 subjects that were untreated and free of CAD at FH diagnosis, 2,892 CAD events would occur if they would remain untreated, whereas 2,045 events (71%) can be prevented if 85% would be treated.

Conclusion: Genetic screening for FH is currently effective in preventing CAD in the Netherlands, with 3 untreated subjects required to be identified with FH to avert CAD in 1.

Late Breaking Session

RESULTS OF FIRST FOLLOW UP STUDY WITH GENE THERAPY WITH ALIPOGENE TIPARVOVEC (AMT-011) IN LIPOPROTEIN LIPASE DEFICIENCY (LPLD)

Daniel Gaudet[1], J. de Wal[2], D. Brisson[3], N. van den Bulk[2], S. Greentree[2], J. Méthot[1]

[1]Dept of medicine, Université de Montréal, Ecogene-21 and Lipid Clinic, Chicoutimi, QC, Canada, [2]Amsterdam Molecular Therapeutics (AMT) B.V., Amsterdam, The Netherlands, [3]Ecogene-21 and Lipid Clinic, Chicoutimi, QC, Canada

Introduction: LPLD is an inherited disorder of lipid metabolism. LPLD results in chylomicronaemia, detectable as severe hypertriglyceridaemia. Recurrent pancreatitis is the most frequent complication, others are diabetes and increased tendency for atherosclerosis.

Aims: The CT-AMT-011-01 study assessed the safety and efficacy of alipogene tiparvovec (AMT-011, Glybera®), which contains LPL^{S447X}, a gain of function LPL-gene mutation, in a non-replicating, non-integrating adeno-associated virus derived vector.

Methods: 14 adult LPLD patients were enrolled in and received $3x10^{11}$ or $1x10^{12}$ gc/kg of alipogene tiparvovec, via a single series of IM leg injections; 12 patients received a 12 weeks' immunosuppressant regime additionally. Patients were evaluated during 12 weeks post administration, with long term follow-up planned for 15 years. Prior to administration patients had been prospectively observed for 3-9 months.

Results: Alipogene tiparvovec was well tolerated, safe and efficacious, with reduction in pancreatitis risk over the first few years now being most noticeable. An >40% reduction in fasting triglyceride (TG) levels was observed in the majority of subjects beginning 2 weeks post administration and maintained during the 12 weeks study. After approximately 4 months, TG trended back towards baseline. We will report data from routine lipoprotein/lipid analysis, which provide explanations for the previously unexpected TG. Persistent gene expression was confirmed in injected muscle tissue after half a year and clinically patients stayed well during 2 years.

Conclusions: Alipogene tiparvovec gene therapy is associated with lasting changes in lipoprotein characteristics and clinical patterns. Observations from our study may add to understanding normal and pathological lipid metabolism.

Poster Session

ADENO-ASSOCIATED VIRUS 9 VECTOR IN THE GENE THERAPY OF OCCLUSIVE VASCULAR DISEASES

Devendra Agrawal[1], D. Pankajakshan[2], M. Del Core[1], I. Pipinos[3], G. Hatzoudis[4]

[1]Clinical & Translational Science, [2]Creighton University School of Medicine, [3]Vascular Surgery, University of Nebraska Medical Center, [4]Surgery, Creighton University School of Medicine, Omaha, NE, USA

The transduction of AAV9 vector containing SM22a promoter with either eGFP or LacZ as reporter genes was analyzed in swine central and peripheral arteries. The AAV9 vectors (1×10^{13} pfu) were administered into coronary and carotid arteries of domestic swine using irrigating balloon catheter-based gene delivery approach. Injury in the arterial endothelial layer was created by balloon inflation with simultaneous delivery of the vector. Segments from all vessels were excised at various time points and immediately frozen for mRNA/protein expression or were processed for frozen sections for X-Gal, GFP or H&E staining. GFP was visualized distinctly in the medial layer. Frozen sections were stained and β-galactosidase gene transfer was assessed by blue-stained cell nuclei. The endothelial and adventitial layers were carefully scraped off from the harvested vessel and total RNA was isolated from the remaining medial layer for qPCR. In the medial layer of coronary and femoral arteries LacZ mRNA expression was visualized 7 days after vector administration. The GFP mRNA expression was detected at 7 days and lasted for at least 2 months showing the prolonged expression of the AAV9-vector. The GFP expression was confirmed by Western blot. Control arteries did not show any expression of GFP or LacZ. There was no significant effect of AAV9 viral transduction on serum amylase and serum CRP levels. These findings support the use of AAV9 as a vector to effectively transduce a gene in coronary arteries.

(Supported by LB692 Tobacco Settlement Funds and Gene Therapy Resource Program (GTRP) of the NIH-NHLBI at UPenn Vector Core for providing the gene vectors)

Poster Session

ANGIOTENSIN CONVERTING ENZYME POLYMORPHISM IN FAMILIAL HYPERCHOLESTEROLEMIC

Francesco Sbrana[1], F. Bigazzi[1], M. Puntoni[2], I. Fossa[1], B. dal Pino[2], D. Pianu[1], D. Iori[1], M.G. Andreassi[1], T. Sampietro[2]

[1]Gabriele Monasterio CNR Regione Toscana Foundation of Pisa, [2]CNR Institute of Clinical Physiology, Pisa, Italy

Background: LDL apheresis is indicated in homozygous familial hypercholesterolemia (FH) patients and in drug refractory familial hypercholesterolemia with coronary heart disease. This therapy usually in well tolerate but can be complicated by bradykinin release, directly activated by LDL apheresis systems. The bradykinin release may develop anaphylactoid reactions with different severity.

Angiotensin Converting Enzyme (ACE) polymorphism, possibly linked to the bradykinin system activation, was investigated in a group of FH patients on LDL apheresis treatment.

Method: The ACE gene I/D polymorphism was detected by PCR in a group of 20 patients with familial hypercholesterolemia complicated by coronary heart disease and regularly treated by LDL apheresis. The ACE gene I/D polymorphism of this patients was correlated with that of a control group of 138 healthy subjects.

Results: The DD variant frequency of ACE gene I/D polymorphism, compared to variants ID and II, was found to be significantly higher ($p < 0.05$) in the group of patients respect to the healthy subjects.

Conclusion: The ACE gene I/D polymorphism analysis in familial hypercholesterolemia may be useful to identify subjects more prone to develop coronary heart disease and those prone to anaphylactoid reactions.

Poster Session

TOWARDS TARGETED IMAGING OF ATHEROSCLEROSIS BY USING PET

Björn Gustafsson, K. Caidahl

Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Current methods for atherosclerosis imaging are invasive and geared towards indirectly imaging the lumen, rather than the lesion itself. We describe syntheses and development of targeted contrast agents that are shown to be taken up by macrophages. The probes are labelled with a fluorescent moiety (FITC) and uptake dependencies are evaluated by Fluorescence-Activated Cell Sorting and also imaged by confocal microscopy. By exchanging the fluorescent moiety with a positron emitting one, this will potentially enable non-invasive *in vivo* Positron Emission Tomography (PET) imaging of early atherosclerosis. We propose to use the high sensitivity of PET in combination with other imaging modalities to locate regions of the inflamed vascular wall. Later these PET-agents could also be conjugated with a paramagnetic metal and probed at high-resolution by using MRI. The worldwide interest in multimodality imaging has surged in recent years and multimodality probes will play a pivotal role in clinical molecular imaging of the future. These agents are targeted to macrophages, an early cellular component of developing plaques. Macrophages are targeted through the scavenger receptors using contrast agents derived from known scavenger receptor ligands. The agents are based on maleylated human serum albumin conjugated with FITC for evaluation of cellular uptake. The 1,4,7-triazacyclononane-1,4,7-triacetic acid ligand system, that can tether metals with positron emitting properties, will later replace the FITC for PET-imaging purposes. Experiments in cultured mouse macrophages demonstrate uptake dependency on concentration, as well as amount of conjugating groups, of the probes. Confocal microscopy images show uptake of probe in the cells.

Poster Session

A NOVEL METHOD OF CLINICAL ULTRASOUND EVALUATION OF VERY EARLY STAGES OF ATHEROSCLEROSIS

Yaroslav Ashikhmin, O. Dikur, O. Drapkina

Cardiology, I.M. Sechenov Moscow Medical Academy, Moscow, Russia

Aim: To develop novel reliable non-invasive method of clinical assessment of very early atherosclerotic lesions.

Patients and methods: We enroll in the study 10 patients with advanced diffuse atherosclerosis; 11 patients with subclinical atherosclerosis, arterial hypertension, and impaired endothelial function (EF); 10 patients with no evidence of atherosclerosis, normal intima-media thickness (IMT) and EF, presented with dyslipidemia, smoking, obesity; and 8 comparable healthy controls. In all of these patients comprehensive clinical evaluation with high-resolution B-mode ultrasound imaging of common carotid artery (CCA) structure and pulse-motion were obtained in uniform regimen by single operator. Then arterial wall images were 10-fold enlarged using fractal-based algorithm. After that shear stress, viscosity, stiffness and dimensions of CCA layers were assessed. Then *slickness* and *echo-heterogeneity* of media (*E-hm*) and endothelium were evaluated by special computed analysis with 3D-reconstruction of arterial wall. Further, comparing data with adventitia reflectivity, "*echo-fibrosis*" indexes were estimated.

Results: Significant and close correlations between the clinical progression of atherosclerosis and novel ultrasound-derived characteristics of CCA media/endothelium, independent of IMT and stiffness increase and EF impairment, were found. Increased *E-hm* was the most sensitive marker of disease progression correlated well with LDL-cholesterol level (r=0.7), cardiovascular risk profile (r=0.7), and permitted to differentiate healthy controls from high-risk patients without overt atherosclerosis. Also, the association of increase of *E-hm* and media thickness with local shear stress and arterial viscosity was shown.

Conclusion: The novel method allows of non-invasive subtle evaluation of CCA intima and media characteristics enable to catch atherosclerotic lesions at the earliest stage.

Poster Session

COMMON CAROTID INTIMA MEDIA THICKNESS IS INDEPENDENTLY ASSOCIATED WITH PERIPHERAL ARTERIAL DISEASE IN UK 1ST GENERATION ASIAN AND BLACK MIGRANTS

Philip Bennett[1], S. Silverman[2], P. Gill[3], G. Lip[1]

[1]Centre for Cardiovascular Sciences, University of Birmingham, [2]Department of Vascular Surgery, Sandwell & West Birmingham Hospitals NHS Trust, [3]School of Health & Population Sciences, University of Birmingham, Birmingham, UK

Introduction: Little is known about common carotid intima media thickness (CCIMT) and its relationship to peripheral arterial disease (PAD) in Black and Indian subcontinent (Asian) 1st generation UK migrants. We compared CCIMT between these ethnic groups and its association with PAD.

Methods: 293 Asian and 199 Black participants aged≥45 were recruited. Mean and maximum CCIMT was measured. PAD was diagnosed using ankle brachial pressure index < 0.9 and Intermittent Claudication (IC) using the Edinburgh Claudication Questionnaire.

Results: Black participants had greater mean but not maximum, CCIMT than Asians in both men (0.67± 0.14mm vs. 0.63± 0.14mm; p=0.04) and women (0.61± 0.13mm vs. 0.58 ±0.12mm; p=0.044). Black race was an independent predictor of both mean (p=0.02) and maximum (p=0.036) CCIMT after adjustment for cardiovascular risk factors. In Asians and Blacks, mean and maximum CCIMT were significantly greater in those with PAD than without (p< 0.05), independent of traditional cardiovascular risk factors. Asians with IC had higher mean and maximum CCIMT than those without (0.82 ± 0.14 vs. 0.61± 0.13mm; p=0.054 and 0.96 ± 0.17 vs.0.73 ± 0.16mm p=0.073 respectively) though this was not significant.

Conclusions: Being Black is an independent predictor of mean and maximum CCIMT adjusting for traditional risk factors. PAD is an independent predictor of both mean and maximum CCIMT adjusting for traditional risk factors and racial group.

Poster Session

IDENTIFICATION AND CHARACTERIZATION OF CORONARY PLAQUES WITH CT VIRTUAL INTRAVASCULAR ENDOSCOPY: A PICTORIAL REVIEW

Thanapong Chaichana, Z. Sun

Imaging and Applied Physics, Curtin University of Technology, Perth, WA, Australia

Purpose: To identify and characterize the coronary plaques, as well as the position of the plaques in relation to the coronary ostium using multislice CT virtual intravascular endoscopy (VIE).

Materials and methods: 20 patients suspected of coronary artery disease undergoing 64-slice CT angiography were included in the study. 3D VIE images were reconstructed to visualise the intraluminal appearance of normal coronary wall, coronary plaques in terms of characterization, location and degree of stenosis. Coronary plaques were characterized into non-calcified, calcified and mixed plaques.

Results: VIE was successfully generated in all of the patients with clear demonstration of the spectrum of intraluminal findings of coronary plaques. Regular and smooth intraluminal appearance was commonly seen with VIE in non-calcified and focally calcified plaques, while irregular luminal changes were noticed in the extensively calcified and mixed plaques. VIE is able to accurately confirm the degree of coronary stenosis without being affected by the blooming artifacts resulting from severe calcification.

Conclusion: VIE provides unique information about intraluminal changes due to presence of coronary plaques. Research findings from this study will provide additional information compared to conventional views, and so accurate assessment of coronary plaques with regard to corresponding coronary luminal changes and prediction of disease progression could be achieved.

Poster Session

MAGNETIC RESONANCE IMAGING OF HIGH RISK ATHEROSCLEROTIC PLAQUES USING GADOFLUORINE M IN A HYPERLIPIDEMIC ANIMAL MODEL AT 3T

Harald Ittrich[1], T. Nielsen[2], K. Peldschus[1], S. Tobias[3], B. Misselwitz[4], H.-J. Weinmann[4], C. Weber[1], G. Adam[1]

[1]Dept. of Diagnostic and Interventional Radiology, University Hospital Hamburg-Eppendorf, [2]Sector Medical Imaging Systems, Philips Research Europe, Hamburg, Germany, [3]Division of Imaging Sciences, School of Medicine, King's College London, London, UK, [4]Research Laboratories, Bayer Schering Pharma AG, Berlin, Germany

Purpose: To evaluate the plaque-enhancing potential, to find the lowest administration dose and to quantify the uptake of Gadofluorine M (GdF) in atherosclerotic plaques in an animal model in MRI at 3T.

Material and methods: The aorta of 12 Watanabe heritable hyperlipidemic (WHHL) and 8 New Zealand White rabbits (NZW, control) was estimated before and after administration (pa) of GdF (4 groups, injection dosages: 100, 50, 25, 12.5 µmol GdF/kg b.w.) in a 3T clinical MRI scanner (Philips Intera) using a 3D IR-TurboFLASH sequence and T1 relaxation Look-Locker (LL) - sequence. Aortic GdF uptake was quantified by SNR measurements. T1 relaxation data analyses were performed using a software tool (Philips Research Labs, Germany). The plaque burden of matched aortic regions was assessed histologically (HE, macrophage antibody RAM11). Statistical differences were tested by student's t-test.

Results: Aortic wall enhancement was detected in WHHL after administration of 100, 50 and 25 µmol GdF ($p < 0.05$). No significant uptake was found in WHHL of the 12.5µmol group and all NZW animals ($p > 0.05$). Histological sections showed a strong correlation between contrast-enhanced aortic regions and inflammatory, lipid-rich plaques of WHHL. NZW animals demonstrated normal aortic layering without plaque formations. R1 relaxation rate (pa) in the WHHL plaques correlated strongly with administered GdF dose ($R^2=0.99$), whereas no significant R1 changes were measured in NZW controls.

Conclusion: Gadofluorine-enhanced MRI improves detection of early, nonstenotic stages in atherosclerosis. The LL sequence allows a quantitative determination of GdF uptake in plaques.

Poster Session

SYNTHESIS, PHYSICOCHEMICAL AND TOXICOLOGICAL CHARACTERIZATION OF ^{59}FE-LABELLED NANOPARTICLES FOR TARGETING ARTERIOSCLEROTIC LESIONS

Barbara Freund[1], A. Giemsa[1], H. Ittrich[2], O. Bruns[3], U. Tromsdorf[4], J. Heeren[1], H. Weller[4], H. Hohenberg[3], G. Adam[2], U. Beisiegel[1], P. Nielsen[1]

[1]Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, [2]Department of Diagnostic and Interventional Radiology, University Medical Center Hamburg, [3]Heinrich-Pette-Institute for Experimental Virology and Immunology, [4]Institute of Physical Chemistry, University Hamburg, Hamburg, Germany

Objective: The detection of early arteriosclerotic lesions remains a challenging task for future diagnostic procedures. The application of specifically designed superparamagnetic nanoparticles (SPIOS) and magnetic resonance imaging technique (MRI) could allow a molecular targeting for this important pathological process. Recent work from our laboratory (Bruns et al. Nature Nanotechnology 2009) has for example used “chylomicron-like” lipoproteins (”nanosomes”) labeled with magnetic or fluorescent nanoparticles to follow pathways of lipid metabolism in mice. One problem in MRI studies is the difficulty to relate MR signals to the unknown local concentrations of SPIOS.

Methods: Following known synthetic strategies, we synthesized crystalline and monodisperse maghemite nanoparticles with a diameter of 11 nm with different hydrophobic and hydrophilic shells.

Results: ^{59}Fe-labelling was introduced by a postsynthetic procedure. Several analytical test methods (size-exclusion chromatography, controlled digestion with iron chelators, mineral acids) demonstrated a stable labelling of the iron oxide core with ^{59}Fe. Pilot in-vivo experiments using hydrophilic ^{59}Fe-NP and ^{59}Fe-NP-labeled nanosomes were performed in mice after i.v. injection.

Conclusions: These early results show already that with radiolabelled NPs the distribution of nanoparticles in vivo can be analyzed more precisely than before. Among the feasible applications, specific MR proton T1 and T2 relaxivities in tissue, organs and lesions from functionalized NP can be measured, and the metabolism and toxicity of iron based NPs can be followed in closer details.

Poster Session

ATHEROSCLEROTIC BURDEN IN ASYMPTOMATIC PATIENTS WITH METABOLIC SYNDROME EVALUATED BY COMPUTED TOMOGRAPHY ANGIOGRAPHY

Marcello Arca[1], G. Pigna[1], F. Zaccagna[2], B. Cavallo Marincola[2], A. Montali[1], L. Iuliano[3], A. Napoli[2], C. Catalano[2]

[1]Department of Clinical and Medical Therapy - Ctr for Atherosclerosis, [2]Department of Radiology, University of Rome La Sapienza, Rome, [3]Internal Medicine Unit - Polo Pontino, University of Rome La Sapienza, Latina, Italy

Introduction: Metabolic Syndrome (MetS) heightens the risk for atherogenesis. A question is if the vascular damage is better predicted by the MetS itself or by the MetS-related risk factors. The 64-slice computed tomography angiography (64-CTA) is a useful tool for detecting atherosclerotic lesions in vivo.

Methods: 64 asymptomatic subjects with MetS (46 men, 18 women; age 55±10.1 yrs; BMI 30.5±3.4) and 52 subjects without MetS (25 men, 27 women; age 59.1±8.6 yrs; BMI 25.2±3.4) underwent 64-CTA examination following a contrast medium dose-saving protocol involving ECG modulation and reduced tube voltage. The diagnosis of MetS was performed according to the ATPIII criteria. The atherosclerotic burden was defined as the presence of lesions in at least one segment of coronary and/or carotid vessels.

Results: The age-adjusted prevalence of coronary (60% vs. 51% respectively; $p< 0.3$) and carotid (29% vs. 33%, respectively; $p< 0.1$) plaques was not significantly different in MetS compared to subjects without MetS. The distribution of severity of stenosis did not differ between the groups. However, when study subjects were stratified according to the number of components of MetS (< 2; 2-3; ≥4), those presenting ≥4 MetS-related factors showed a significantly increased prevalence of coronary and carotid lesions compared to the other groups (44,6%, 52% 82.6%, $p< 0.012$ for trend). Subjects with≥4 MetS-related factors showed the worst metabolic profile.

Conclusions: These results suggest that in MetS patients the atherosclerosis burden is more strongly associated to the number of MetS-related factors than to the clinical diagnosis of MetS per se.

Poster Session

TARGETED STERICALLY STABILIZED LIPOSOMES FOR DIAGNOSTIC IMAGING OF ATHEROSCLEROTIC PLAQUES

Ruth Prassl[1], G. Almer[2], M. Saba-Lepek[1], D. Frascione[1], J. Kellner[3], A. Gries[3], C. Diwoky[4], R. Stollberger[4], H. Mangge[2]

[1]Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, [2]Clinical Institute for Medical and Chemical Diagnosis, [3]Institute of Physiology, Medical University Graz, [4]Institute of Medical Engineering, University of Technology Graz, Graz, Austria

We have designed polyethylene glycol (PEG-) coated (sterically stabilized) liposomes to carry high payloads of imaging reagents such as paramagnetic contrast agents (Gd-based), ironoxide nanoparticles or optical active compounds (e.g. fluorescent dyes) to cells with the goal to improve non-invasive molecular imaging modalities by specific targeting. To target atherosclerotic plaques we have covalently coupled selected biomarkers to the distal ends of the PEG-chains, which are located at the surface of the liposomes. These functionalized liposome constructs were characterized by photon correlation spectroscopy, modified native gel electrophoresis and Western Blot. As targeting sequences we have chosen interleukin-10 (IL-10), the globular domain of adiponectin (gAcrp30) and anti-LOX-1 mAB. These biomarkers bind to atherosclerotic aortas of ApoE-deficient mice as shown by *ex vivo* imaging using confocal laser-scanning microscopy (CLSM). For the ligand-conjugated liposomes we found a pronounced signal enhancement ex vivo, whereas no signal was detected in less injured aortic surfaces or in arteries of WT-mice. With IL-10-targeted liposomes we already observed a strong *in vivo* staining signal with CLSM. Now, Gd-DTPA-lipid and ultra small ironoxide nanoparticle (USPIOs) containing targeted liposomes are established to improve image sensitivity for *in vivo* magnetic resonance imaging (MRI). A contrast enhancement in terms of T_1-relaxivity or in hypotense T_2-signals could be achieved for paramagnetic or magneto-liposomes, respectively. In conclusion, the combinatory use of specific anti-inflammatory biomarkers as targeting sequences and multiple or high payloads of contrast agents within one liposome particle opens the opportunity for early recognition, differentiation and visualization of unstable vulnerable atherosclerotic plaques by imaging.

Poster Session

WHHLMI RABBIT IS AN ANIMAL MODEL FOR ANGINA AND/OR CORONARY SPASMS

T. Kobayashi[1], T. Ito[1], S. Yamada[1], N. Hirayama[1], K. Hirata[2], T. Ishida[2], **Masashi Shiomi**[1]

[1]Institute for Experimental Animals, [2]Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Purpose: Coronary spasms are one of the important causes of myocardial ischemia. However, the mechanisms of coronary spasms and the highly susceptible regions are still unknown because of the lack of suitable animal models for the disease. Therefore, we are trying to develop an animal model for coronary spasms and/or angina.

Methods: Coronary atherosclerosis and myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits were used in this study. Under anesthesia with intravenous injection of ketamine plus midazolam, WHHLMI rabbits were administered intravenously with serotonin, ergonovine, dobutamine, norepinephrin, or angiotensin-II. ECG and blood pressure at the femoral artery were recorded during the experiment. After examination of ECG, the coronary atherosclerosis was examined histopathologically.

Results: The blood pressure was raised by more than 50 mmHg with intravenous injection of each drug. ECG documented depression of the ST segment, reduction of R-wave amplitude, or T-inversion. Ventricular arrhythmia was also observed after bolus injection of serotonin or ergonovine in combination with the perfusion of dobutamine, norepinephrin or angiotensin II. These ECG changes were correlated with the degree of coronary atherosclerosis.

Conclusions: WHHLMI rabbits will be an useful animal model for angina in which coronary spasm and subsequent myocardial ischemia can be pharmacologically evoked in vivo.

Poster Session

PREVENTION OF ACTIVATED MACROPHAGE-INDUCED ADHESION MOLECULES BY CHLORELLA EXTRACT IN ENDOTHELIAL SEVC CELLS

M.-F. Shih[1], **Jong-Yuh Cherng**[2]

[1]Pharmacy, Chia-Nan University of Pharmacy & Science, Tainan, [2]Biochemistry & Chemistry, National Chung Cheng University, Chia-Yi, Taiwan R.O.C.

The inflammatory response in large vessels involves the up-regulation of vascular adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM-1), and E-selectin. Inflammatory cytokines such as TNF-α, IL-1, or IL-6 are thought to play important roles in the development of atherosclerosis. Chlorella has been shown to lower high fat diet-induced atherosclerosis. In addition, we have previously shown that partial purified lipophilic chlorella extract (PPLEC) possess strong anti-inflammatory effect. The aim of this study is to investigate the possible preventing role of PPLEC on pro-inflammatory cytokine-induced expression of vascular adhesion molecules.

Endothelial cells (SEVC cell line) were treated with conditioned culture media (normal culture media contains 50% of LPS-activated macrophage culture media, in which there contained TNF-α, IL-1, and IL-6) with or without high (0.5 mg/ml) or low (0.125 mg/ml) dose of PPLEC extracts. Indomethacin (0.25 μM) was used as a positive control. Production of VCAM-1, ICAM-1 and E-selectin was measured by ELISA assay kits.

Production of ICAM, VCAM or E-selectin was all increased by additional RAW culture media in SEVC cells. The induction of E-selectin and ICAM was significantly prevented by both high and low doses of PPLEC or indomethacin treatment. However, the prevention of VCAM production was only seen in high dose of PPLEC.

PPLEC not only possess anti-inflammatory effect (previous study) but also prevents pro-inflammatory cytokines-induced adhesion molecule production in endothelia. These data indicate that PPLEC can be a potential material to develop in preventing chronic inflammatory-related diseases.

Poster Session

CILOSTAZOL AMELIORATES METABOLIC ABNORMALITIES IN A DB/DB MOUSE MODEL OF TYPE 2 DIABETES VIA ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORΓ TRANSCRIPTION

So Youn Park, K.W. Hong, W.S. Lee, C.D. Kim

Medical Research Center for Ischemic Tissue Regeneration; Department of Pharmacology, Pusan National University, Gyeongnam, Republic of Korea

This study evaluated the *in vivo* efficacy of cilostazol to protect a db/db mouse model of Type 2 diabetes against altered metabolic abnormalities and pro-inflammatory markers via activation of PPARγ transcription. Eight-week old db/db mice were treated with cilostazol or rosiglitazone for 12 days. Cilostazol significantly decreased plasma glucose and triglyceride levels, as did rosiglitazone, a PPARγ agonist. Elevated plasma insulin and resistin levels were significantly decreased by cilostazol, and decreased adiponectin mRNA expression was elevated along with increased plasma adiponectin. Cilostazol significantly increased both adipocyte fatty acid binding protein (aP2) and fatty acid transport protein (FATP-1) mRNA expressions with increased glucose transport 4 in the adipose tissue.

Cilostazol and rosiglitazone significantly suppressed pro-inflammatory markers (superoxide, TNF-α and vascular cell adhesion molecule-1) in the carotid artery of db/db mice. In *in vitro* study with 3T3-L1 fibroblasts, cilostazol significantly increased PPARγ transcription activity, as did rosiglitazone. The transcription activity stimulated by cilostazol was attenuated by KT5720, a cAMP-dependent protein kinase inhibitor, and GW9662, an antagonist of PPARγ activity, indicative of implication of PI3-k/Akt signal pathway. These results suggest that cilostazol may improve insulin sensitivity along with anti-inflammatory effects in Type 2 diabetic patients via activation of both cAMP-dependent protein kinase and PPARγ transcription.

Poster Session

PREVENTION OF DOCA/SALT HYPERTENSION-INDUCED RAT RENAL INJURY BY ANTIOXIDANT THERAPY

Mehri Kadkhodaee[1], B. Seifi[1], S.M. Karimian[1], M. Zahmatkesh[1], E. Bakhshi[2]

[1]Physiology, [2]Biostatistics, Tehran Medical Sciences University, Tehran, Iran

Introduction: Hypertension is a major cardiovascular risk factor and a contributor to End Stage Renal Disease. This study examined whether the antioxidant therapy with vitamin E or C, could modify renal damage and high blood pressure in DOCA-salt induced hypertension.

Methods: One week after uninephrectomy, rats in the DOCA-salt group treated 5 times a week with DOCA suspended in oil, which were administered subcutaneously for 4 weeks (25 mg/kg). 1% NaCl + 0.2% KCl were added to their water for drinking. Sham rats were received oil, 5 times a week, subcutaneously. In the two other groups vitamin E (200 mg/kg/day/gavage) or vitamin C (200 mg/kg/day/gavage) were co-administered with DOCA-salt for 4 weeks. Systolic blood pressure (SBP) was measured by the tail-cuff method. Levels of renal antioxidants, renal damage indices and histological changes were studied in all groups.

Results: DOCA-salt treated rats exhibited marked increase in blood pressure compared to that in sham group (183.57 ± 6.24 vs 109.28 ± 2.97 mmHg). Levels of urinary N-acetyl glucoaminidase) NAG and protein excretion were significantly increased. Decreased renal reduced glutathione (GSH) contents and ferric reducing ability of plasma (FRAP) were demonstrated in DOCA-salt treatment as well as histological changes. Treatment with vitamin C or vitamin E for 4 weeks preserved the renal antioxidant levels and prevented renal damage and elevation of blood pressure in the DOCA-salt treatment.

Conclusions: Antioxidant therapy decreased renal damage in DOCA-salt treated rats. These data suggests a role for oxidative stress in the development of nephropathy in DOCA-salt hypertension.

Poster Session

SERUM LEVELS OF LEPTIN AND LIPID PROFILES FOLLOWING LOVASTATIN THERAPY IN DIABETIC NEPHROPATHY

Nadereh Rashtchizadeh[1], A. Ghorbanihaghjo[2], H. Argani[3], J. Safa[3], M. Rahbaninoubar[1]

[1]Clinical Biochemistry, Tabriz University of Medical Sciences-Drug Applied Research Center-Tabriz-Iran, [2]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [3]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction: Diabetes mellitus is very widespread disease with disabling and potentially fetal consequences. Controlling metabolic derangements is a great challenge in diabetic nephropathy of which, lipid profile is of special importance. It is proposed that serum Leptin is an important factor in this regard and it might be decreased by statin. The study designed to evaluate the serum leptin level and lipid profiles in diabetic nephropathy following Lovastatin administration.

Methods & materials: Thirty patients with definite diagnosis of diabetic nephropathy, with mean age of 54±6 years referred to department of nephrology in Tabriz Emam Hospital were selected. Oral Lovastatin, 20 mg tablet per day for 3 months administered to all patients. Serum lipid profile (Cholesterol, HDL-C, LDL-C, and Triglyceride) and Leptin levels were determined and compared to the baseline results. Serum Leptin was measured by ELISA and lipids and lipoproteins by standard methods.

Results: After the treatment the mean levels of Cholesterol (199.00±43.33 mg/dl vs. 164.67±35.19 mg/dl; p< 0.001), LDL-C (116.16±46.54 mg/dl vs. 84.48±29.23 mg/dl; p< 0.001) and Leptin (10.78±8.30 ng/ml vs. 7.80±5.41ng/ml; p=0.006) significantly decreased and that of serum HDL-C (40.00±4.31 mg/dl vs. 42.80±5.15 mg/dl; p=0.005) markedly increased. No significant changes observed in the mean levels of Triglyceride (175.37±94.98 mg/dl vs. 152.07±94.73 mg/dl; p=0.156).

Conclusion: The study showed that administration of Lovastatin altering the lipids and lipoproteins levels could significantly decrease the mean level of serum Leptin, in patients with diabetic nephropathy. Further studies should be undertaken to evaluate the prognostic value of leptin in the diabetic nephropathy.

Poster Session

AEROBIC TRAINING REDUCED LEPTIN LEVELS IN ADOLESCENTS WITH DOWN SYNDROME

Francisco Javier Ordonez[1], I. Rosety[2], M.A. Rosety[2], A. Camacho[3], G. Fornieles[2], M. Bernardi[4], M. Rosety-Rodriguez[1]

[1]Sport Medicine, [2]University of Cadiz, Cadiz, [3]Internal Medicine, Hospital Juan Ramon Jimenez, Huelva, Spain, [4]Sport Medicine, Universita La Sapienza, Roma, Italy

Background and aims: It is widely accepted adipokines such as leptins have been implicated in the pathophysiology of obesity. A recent study have also concluded leptin levels were higher in prepuberal children with Down syndrome when compared to unaffected siblings, that may explain at least in part, their high risk for obesity. Fortunately regular exercise may reduce leptin levels in children without trisomy 21. Accordingly the present study was designed to assess the influence of aerobic training on leptin levels in adolescents with Down syndrome.

Methods: To get this goal 31-adolescents with Down syndrome (16.3±1.1 years; 155.2±5.7 cm; 70.8±4.5 kg) performed a 12-week training program in treadmill with 3 days/week, consisting of warm-up (15min), main part (20-35min) at a work intensity of 60-75% of peak heart rate (HRmax=194.5-[0.56 age]) and cool-down (10min). Control group included 7 age, sex and BMI-matched adolescents with trisomy 21 that did not perform any training program. Leptin levels were determined via double-antibody radioimmunoassay (Esoterix laboratories, Los Angeles, CA) 72-hours before starting the protocol (pre-test) and after its ending (post-test). Further our protocol was approved by an institutional ethic committee.

Results: When compared to baseline leptin levels were reduced significantly after our 12-week protocol (30.6±2.8 vs 24.8±2.5 ng/ml; $p < 0.05$). No changes were reported in controls.

Conclusion: It was concluded a 12-week aerobic training program reduced plasmatic leptin levels in adolescents with Down syndrome. Further long-term follow-up studies are required to determine whether correction of this adipokine improves clinical outcomes of individuals with trisomy 21.

Poster Session

ARTERIAL STRUCTION AND FUNCTION IN FAMILIAL HYPERCHOLESTEROLEMIA: A META-ANALYSIS OF CASE-CONTROL STUDIES

Constantina Masoura, K. Aznaouridis, C. Pitsavos, I. Skoumas, C. Vlachopoulos, C. Stefanadis

1st Department of Cardiology of Athens Madical School- Hippocration Hospital, Athens, Greece

Introduction: Familial hypercholesterolemia (FH) is an inherited disorder associated with early atherosclerotic disease. Flow-mediated dilation of the brachial artery (FMD, a measure of endothelial function) and carotid and femoral intima-media thickness (IMT, a measure of subclinical atherosclerosis) comprise predictors of cardiovascular risk.

Objectives: We conducted this research to calculate quantitative estimates for the impact of FH on FMD and carotid and femoral IMT.

Methods: We meta-analyzed 8 case-control studies with data on FMD, 17 studies (19 cohorts) on carotid IMT and 4 studies (5 cohorts) on femoral IMT in untreated FH patients and controls.

Results: FH had lower FMD compared with controls [pooled weighted mean difference (WMD) of FMD: -1.24% (95% CI -1.66 to -0.82%, P< 0.001). Both carotid and femoral IMT were significantly higher in FH patients [pooled WMDs of 0.78 mm (95% CI 0.57 to 0.98 mm), P< 0.001; and 0.67 mm (95% CI 0.36 to 0.97 mm, P< 0.001 respectively, figures). Meta-regression analysis showed an inverse relationship between age and FMD difference (p< 0.05), indicating a higher impairment of FMD at young FH compared to controls of similar age. We observed significant associations of the WMD of carotid and femoral IMT with the difference of total cholesterol between FH and controls (both P< 0.05), indicating a potential role of lipid levels in subclinical atherosclerosis in FH.

Conclusions: FH have impaired endothelial function and a higher degree of subclinical atherosclerosis compared with age-matched controls. These findings may explain the high cardiovascular risk observed in patients with FH.

Poster Session

COMPARISON OF ADIPOSE TISSUE GENE EXPRESSION OF ADIPONECTIN, TUMOR NECROSIS FACTOR-A AND LEPTIN IN PATIENTS WITH AND WITHOUT METABOLIC SYNDROME

Selçuk Görmez[1], A. Demirkan[2], B. Çaynak[3], F. Atalar[4], D. Günay[5], B. Akpınar[3], U. Özbek[4], V. Aytekin[6], N. Yazıcıoğlu[1], A.S. Büyükdevrim[7]

[1]Department of Cardiology, Florence Nightingale Hospital, Istanbul, Turkey, [2]Department of Genetic Epidemiology, Erasmus MC, Rotterdam, Rotterdam, The Netherlands, [3]Department of Cardiovascular Surgery, Istanbul Bilim University, Faculty of Medicine, [4]Department of Genetics, Istanbul University, Institute for Experimental Medical Research, [5]Departmet of Biochemistry, Florence Nightingale Hospital, [6]Department of Cardiology, Istanbul Bilim University, Faculty of Medicine, [7]Turkish Diabetes Consortium, Istanbul, Turkey

Objective: Metabolic syndrome (MS) is associated with an increased risk of coronary heart disease (CAD) and type 2 diabetes mellitus (DM). In MS, adipose tissue was shown to function as both a paracrine and endocrine organ and to secrete adipocytokines from the visceral fat, especially. In the current study, MS patients were compared to the non-MS patients in terms of protective and proinflammatory adipocytokines secretion from the visceral and parietal adipose tissue as adiponectin, tumor necrosis factor-α (TNF-α) and leptin gene expression levels.

Methods: 37 patients with MS who were to undergo coronary bypass surgery due to CAD (MS group) and 23 non-MS patients without CAD who were to undergo heart valve surgery (control group) in our institution were recruited prospectively to our study. Relative gene expressions of adipocytokines (arbitrary unit) of two groups in epicardial, pericardial and subcutaneous adipose tissue were evaluated by using quantitative RT-PCR method.

Results: Adiponectin expression of epicardial and pericardial adipose tissue were significantly lower in MS group compared to the control group ($p< 0.0001$, $p=0.04$, respectively) while subcutaneous adiponectin gene expressions did not differ significantly ($p=0.64$). TNF- α and leptin expressions were found higher with a statistical significance in all the epicardial, pericardial and subcutaneous adipose tissues of the MS group ($p< 0.0001$).

Conclusion: TNF-α and leptin expression increases prominently in the epicardial, pericardial and subcutaneous adipose tissues of MS patients with CAD. However, adiponectin expression decreases in epicardial and pericardial adipose tissue.

Poster Session

ORPHAN NUCLEAR RECEPTOR NUR77 SUPPRESSES INFLAMMATORY RESPONSE PARTIALLY DEPENDENT ON COX-2 IN MACROPHAGES INDUCED BY OXIDIZED LOW-DENSITY LIPOPROTEIN

Shao Qin, S. Linghong, H. Ben

Cardiology Department, Ren Ji Hospital, Medical School of Shanghai Jiao Tong University, Shanghai, China

Oxidized low-density lipoprotein (oxLDL) cross-talks with macrophages, and both play a crucial role in the initiation and progression of atherosclerosis. Orphan nuclear receptor Nur77 is potently induced in macrophages by diverse stimuli, suggesting that it may be a key regulator of inflammation in vascular cells. In this study, we investigated the detailed mechanism of Nur77 activation and subsequent function in macrophages induced by oxLDL.

We have demonstrated that Nur77 is upregulated in macrophages by oxLDL stimulation, as detected by real-time PCR and Western blotting. We have presented a novel mechanism that oxLDL-induced Nur77 expression is involved in p38 MAPK signal pathway. In activated macrophages, overexpression of Nur77 has been observed to attenuate proinflammatory cytokines and chemokines secretion including tumor necrosis factor (TNF)a and monocyte chemoattractant protein-1(MCP-1). While knockdown Nur77 expression by specific small interfering RNA (siRNA) or overexpression of mutants of Nur77, which lacks its transactivation domains(TAD) and DNA binding domains(DBD) have been found to enhance the secretion. We have also presented a novel mechanism through which Nur77 could negatively regulate inflammatory response by suppressing cyclooxygenase-2 (COX-2) expression in oxLDL-induced macrophages. However, the inhibition of COX-2, Nur77 activation could further reduce MCP-1 and TNFa mRNA level.

Taken together, Nur77 is induced by oxLDL via p38 MAPK signal pathway and subsequently protects against inflammation by the inhibition of proinflammatory COX-2 pathway in activated macrophages, both TAD and DBD domains are required for its function. This may represent a potential molecular target for the prevention and treatment of atherosclerosis.

Poster Session

PLATELET COUNT IS INDEPENDENTLY ASSOCIATED TO ASYMPTOMATIC PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

J. Mancera[1], F. Paniagua-Gómez[1], A. Baca-Osorio[1], L. Ginel-Mendoza[1], M.L. Fernández-Tapia[1], P. González-Santos[2], **Pedro Valdivielso**[2]

[1]Servicio Andaluz de Salud (CS Ciudad Jardín), [2]University of Malaga, Malaga, Spain

Aim: To investigate whether hematological parameters are associated to peripheral arterial disease (PAD) in ambulatory patients suffering from type 2 diabetes (T2D).

Patients and methods: 456 subjects with T2D and aged 50 to 80 years-old were recruited from a single primary care center. Age, sex, prior vascular diseases and medication, anthropometrical data, fasting blood samples and ankle-brachial index (ABI) were assessed from all the participants. Peripheral arterial disease were considered present when ABI < 0.9. Fasting lipids, glycemia, serum creatinine, glycated haemoglobin, microalbuminuria, uric acid, haemoglobin, leukocytes, platelet count were also measured. Multiple logistic regression analyses was built in order to assess the parametrs independently associated to PAD.

Results: 243 men and 213 women were included; they were 61 ± 6 years-old. Among 456 patients, 126 (27%) had PAD according to ABI. In univariate analyses, smoking, hypertension, hypotensive drugs, microvascular disease, body-mass index, duration of diabetes, total cholesterol, fasting triglycerides and platelets were associated to PAD. However, in multivariate analyses, only platelet count, smoking, duration of diabetes and fasting triglycerides were independently associated to PAD. No association was found with age, sex, HDL cholesterol , leukocytes, or lipid-lowering drugs.

Conclusion: Platelet count was the unique hematological parameter associated to subclinical PAD in patients with T2D.

Poster Session

CAROTID-FEMORAL ARTERIAL INDEX AND SEVERITY OF CORONARY ATHEROSCLEROSIS

Mahmoud Soliman[1], H. Hassan[2], T. Gazy[2], W. Fareed[2], A. Reda[2], S. Shalaby[2]

[1]Cardiology, [2]Menofyia, Shebin El Kom, Egypt

Intruduction: Examination of more vascular beds may have more impact than examination of carotids alone.

Aim: Detection of coronary atherosclerosis and assessment of its severity non-invasively by examination of the carotid and superficial femoral arteries.

Methods & results: This study included 120 patients; (30 with normal coronary arteries, group 1, 30 patients with single vessel disease group 2, 30 with two vessel disease group 3, 30 with three vessel disease group 4). The severity of coronary atherosclerosis calculated according to Gensini score.

All patients underwent duplex scaning of the right and left carotid and the right and left femoral superficial arteries.

The Carotid femoral index(CARFEM) in mm was determined as follows: (Intima media thickness of right common carotid + IMT left common carotid + Total width of right femoral + Total Width left femoral) / 4.

The CARFEM index was:0.54+-0.06 in group 1, 0.87+-0.04 in group 2,1.22+-0.08 in group 3 and 1.54+-0.1 in group 4 and the deference was highly significant, P value < 0.001 & also there was highly significant difference between all groups as regard to Gensini score, P value < 0.001., Correlation between CARFEM index & Gensini score among patients with coronary atherosclerosis (groupes 2, 3 & 4) was positive & significant in group 4, r : 0.4 & P value < 0.05.

Conclusion: CARFEM index can differentiate between patients with normal and atherosclerotic coronary arteries, also it can discriminate the severity & extent of coronary atherosclerosis.

Poster Session

ASCENDING AORTA ATHEROSCLEROSIS IS INDEPENDENT OF CAROTID AND FEMORAL ARTERIES ATHEROSCLEROTIC INVOLVEMENT

Mohammad Kazem Tarzamni[1], N. Nezami[2], A. Afrasiabi[3], A.T. Eftekharsadat[4], N. Eshragi[1]

[1]Department of Radiology, Tabriz University (Medical Sciences), [2]Young Researchers Club, Tabriz Islamic Azad University, [3]Department of Cardiovascular Surgery, [4]Department of Pathology, Tabriz University (Medical Sciences), Tabriz, Iran

Objectives: The aim of the present study was to examine the association between carotid and femoral arteries intima media thickness (IMT) and the severity of coronary artery disease (CAD) as well as the atherosclerotic grade of ascending aorta.

Patients and methods: One hundred five candidates of coronary artery bypass grafting (CABG) were evaluated during present cross-sectional study from Aug 2007 to Apr 2009. Prior to CABG, patients underwent carotid and femoral arteries IMT assessment using Hitachi ultrasound machine. A full thickness piece of ascending aorta punched during anastomosis of new vessels shunt used to grade atherosclerosis by microscopy using American heart association classification.

Results: Among 105 CABG candidates, 74.3% and 25.7% were male and female, respectively. Mean age of patients was 57.11±10.26 years. Frequency of coronary vessels involved was 6.7, 28.6 and 64.8 % for one, two and three vessels diseases. 3.8%, 10.5, 20, 32.4, 21 and 12.4 % of patients had respectively negative, grade one, two, three, four, five aorta atherosclerosis. Mean carotid and femoral arteries IMT were respectively 1.77±0.45 and 1.21±0.34 mm. There was not correlation between ascending aorta atherosclerosis grade and carotid or femoral arteries IMT, and carotid and femoral arteries IMT were not different in patients with various grades of aorta atherosclerosis. Also no correlation was found between number of involved coronary artery and carotid or femoral arteries IMT.

Conclusions: Carotid and femoral arteries atherosclerotic involvement and development were begun from early stage of disease and progress independently from aorta atherosclerosis and even coronary artery disease.

Poster Session

NONINVASIVE DETECTION AND EVALUATION OF CORONARY ARTERY PLAQUES WITH MULTISLICE COMPUTED TOMOGRAPHY CORONARY ANGIOGRAPHY IN DIABETIC PATIENTS

Fatma Aboul-Enein

Alexandria University, Alexandria, Egypt

Introduction: Coronary artery disease (CAD) is the leading cause of morbidity and mortality in diabetics (DM). It is unclear whether the coronary atherosclerotic plaque burden is similar in DM as in the general.

Aim of the study was to assess differences in the presence, extent, and composition of coronary atherosclerotic plaque burden as detected by Multislice computed tomography coronary angiography (MSCTA) in DM.

Methods: 150 consecutive patients 69 (46%) were DM underwent MSCTA. Study were classified as having no CAD, mild-moderate CAD (< 50% stenosis), or moderate-severe CAD (>50% stenosis). Plaques were classified as calcific non calcific (mixed) or soft. Finally patients were classified as No CAD, single vessel CAD and >1vessel CAD.

Results: DM less frequently had a normal MSCTA (36.2 % vs 59.3% P=0.008) and more frequently had obstructive plaques (43.5% vs 18.5 p=0.002). They had more calcific (0.83± 1.2 vs 0.28 ±0.71, p=0.004) and non calcific plaque (0.55± 0.88 0.29± 0.52 p=0.048) and had more extensive disease (1.9± 1.8 vs .99± 1.6, p= 0.001). Diabetes correlated with number of obstructive plaques (CI=1.6-7.1, p=0.001), number of calcified plaque (CI= 0.87- 0.54, p=0.001) and presence of multivessel disease (CI=2.41-10.27 p=0.001).

Conclusion: Diabetics have an overall increased coronary atherosclerotic plaque burden with approximately 4-fold higher risk of coronary stenosis independent of other cardiovascular risk factors and a 9 fold higher risk of multivessel CAD independent of other cardiovascular risk factors. Thus, MSCT may be used to identify differences in coronary plaque burden, which may be useful for risk stratification.

Poster Session

EFFECT OF TELMISARTAN ON THE ARTERIOVENOUS INDEX IN RETINAL MICROVASCULARIZATION

José Antonio Díaz Peromingo[1], J.M. Fernández Villaverde[2], A. Carballo Vicente[2], I. Echebarrieta Gorostiaga[3], G. Múgico Sánchez[2], V. Parga García[2], M.A. Pérez Llamas[4], M. Rodríguez Blanco[5], M. Pena Seijo[6], A. Pose Reino[6]

[1]Internal Medicine, Hospital da Barbanza, [2]Family Medicine, Centro de Saúde, Riveira, [3]Family Medicine, Centro de Saúde, Porto do Son, [4]Family Medicine, Centro de Saúde, Pobra do Caramiñal, [5]Oftalmology, [6]Internal Medicine, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

Introduction: Modifications in retinal microvascularization correlate with cerebral, myocardial, renal and peripheral blood flow. Regression of these changes could be evaluated by studying the changes in the arteriovenous (AV) index.

Methods: A total of 45 patients with known hypertension were included. Treatment with telmisartan was started and hydrochlorotiazide was added when needed. A digital retinography was performed at baseline and after 6 months. AV index was calculated using the linear method (semiautomatic). Statistical analysis was performed using a T test for repeated measures and to compare different groups when needed.

Results: 24 patients were women and 21 men. Middle age was 54 (range 21-78) years old. To control blood pressure 6 patients needed hidrochlorotiazide. There were no statistical differences in the venous diameter in both eyes after 6 months of treatment. Mean arterial diameter increased significantly in both eyes (RE 6.01749/6.21876; p=0.001. LE 6.18439/6.32885; p=0.001). The AV index also increased (RE 0.76398/0.81629, p=0.003; LE 0.78361/0.82976, p=0.001). There were no statistical differences regarding to sex and age groups.

Conclusions:

1. Telmisartan ± hidrochlorotiazide improves AV index after 6 months of treatment.
2. There are no statistical differences respecting to sex and age groups in our study.
3. Beyond controlling blood pressure, improving retinal microvascularization could help in reducing cardiovascular risk in hypertensive patients.

Poster Session

PHOTOTHERMAL NANODESTRUCTION VERSUS STROMA ABLATION WITH LENTIVIRAL VECTOR EXPRESSING DIPHTHERIA TOXIN FOR THE MANAGEMENT OF ATHEROSCLEROTIC PLAQUE

Alexandr Kharlamov, Jan L. Gabinsky

Nanobiotechnology, Urals Institute of Cardiology, Yekaterinburg, Russia

Background: Some modern angioplasty techniques generally just manipulate the form of the plaque.

Objective: We designed it to compare two nanobiotechniques of atherodestruction.

Methods: A total of 27 white transgenic swines were assigned to the three groups (silica-gold nanoshells with near-infrared irradiation, lentivirus, and saline control). The stroma-specified lentivirus vector was constructed on the basis of long-term fibroblasts cell culture. An ultrasound-mediated microbubbles system has used for the delivery into carotid arteries. The primary combined outcome was the plaque volume (PV) and the atherothrombosis rate.

Results: A change of the PV (mm^3; on data of IVUS) immediately after the laser irradiation/ in 3 months in the group with using of nanoshells (1st group) was 18.1%/ 34.4% ($p< 0.01$ for all comparisons [FAC]), and in the group with stroma ablation reached 11.6%/ 44.5% ($p< 0.05$ FAC), and in the saline control assessed as 2.9%/ 4.3% ($p< 0.01$ as compared with another groups). There were some cases of atherothrombosis (3 cases) but in the nanoshell group only. The histological analysis has confirmed the 'burning' effect of nanophotodestruction with multiple ruptures, necrosis, and consequent possible functional insufficiency of vessel. In the toxin ablation group we have seen only a degradation of adventitia with signs of the plaque rebuilding, revascularization and degradation of the plaque nucleus.

Conclusions: The toxin ablation technique with viral vectors is more effective as a tool for the angioplasty with more high level of safety opening new opportunities for the following studies and reflex a clinical relevance of this idea.

Poster Session

MODULATION OF ANRIL EXPRESSION MAY MEDIATE THE ASSOCIATION OF CHROMOSOME 9P21 VARIANTS WITH CORONARY ARTERY DISEASE AND STROKE

Michael Cunnington[1], M. Santibanez Koref[1], B. Mayosi[2], J. Burn[1], B. Keavney[1]

[1]Newcastle University, Newcastle upon Tyne, UK, [2]University of Cape Town, Cape Town, South Africa

Introduction: Single nucleotide polymorphisms (SNPs) on chromosome 9p21 are associated with coronary artery disease and stroke. The mechanisms mediating the association are unknown, but risk SNPs are mainly non-coding and may influence gene expression.

Aim: To investigate whether 9p21 SNPs are associated with expression of the three nearest genes; the cell-cycle inhibitors CDKN2A/2B and a non-coding RNA of unknown function, ANRIL.

Methods: We examined association between 56 SNPs in the region and peripheral blood expression of CDKN2A/B and ANRIL in 495 healthy volunteers, using allelic and total expression. Association between SNPs and expression was assessed using likelihood ratio tests.

Results: Total expression of the three genes was correlated ($P< 0.05$), suggesting they are co-regulated. Allelic expression was also correlated ($P< 0.05$), suggesting shared *cis*-acting elements. SNP effects mapped by allelic and total expression were similar ($r=0.96$, $P=2x10^{-92}$), but the power to detect effects was greater for allelic expression. The proportion of expression variance attributable to *cis*-acting effects was 8% for CDKN2A, 5% for CDKN2B, and 20% for ANRIL. Multiple SNPs were independently associated with expression of each gene ($P< 0.01$), suggesting that several sites may modulate disease susceptibility. Risk SNPs were all highly associated ($P< 1x10^{-7}$) with up to 1.9-fold reduction in ANRIL expression, whilst association with the other two genes was only detectable for some. SNPs had an inverse effect on ANRIL and CDKN2B expression, suggesting a possible role of ANRIL in CDKN2B regulation.

Conclusions: Modulation of ANRIL expression may mediate susceptibility to atherosclerosis.

Poster Session

NEW FINDINGS SUPPORTING PAPP-A ROLE IN ATHEROSCLEROTIC PLAQUE DESTABILIZATION

Tatiana Solovyeva[1], A. Postnikov[1], D. Serebryanaya[2], A. Kharitonov[2], A. Arutyunov[3], A. Katrukha[1]

[1]R&D, HyTest, Turku, Finland, [2]Biological, Moscow State University, [3]Pirogov Russian State Medical University, Moscow, Russia

Pregnancy-associated plasma protein-A exists in two isoforms: heterotetrameric 2:2 complex PAPP-A/proMBP (htPAPP-A) found in blood of pregnant women and homodimeric form (dPAPP-A) that was found recently in unstable atherosclerotic plaques. It was shown that levels of dPAPP-A are increased in the blood of ACS patients. It was suggested that dPAPP-A is a new blood marker of acute coronary syndrome (ACS). But the causes of increase of dPAPP-A levels are still unknown. It was supposed that dPAPP-A cleaves IGFBP-4 and enhances local IGF bioavailability that promotes inflammation in atherosclerotic plaques and its destabilization. However specific protease activity of atherosclerotic PAPP-A is still not experimentally shown.

The aim of the current study was to investigate enzymatic properties of dPAPP-A from human atherosclerotic plaques. We have elaborated a method of dPAPP-A isolation from human atherosclerotic tissue using affinity chromatography utilizing MAb 4G11 (HyTest, Finland). Identity this atherosclerotic PAPP-A with recombinant dPAPP-A and htPAPP-A was confirmed by mass spectrometry analysis. Using Western blotting analysis and sandwich immunoassay method we have demonstrated the presence of dPAPP-A and the absence of htPAPP-A in atherosclerotic plaques. Using immunohistochemistry analysis with MAb PAPP30 specific to dimeric form of PAPP-A we have confirmed that only dPAPP-A is expressed in atherosclerotic plaques. Moreover we have shown that atherosclerotic dPAPP-A is an active protease that specifically cleaves IGFBP-4 in the presence of IGF-2 in vitro.

Our findings support the hypothesis that enzymatically active dPAPP-A can participate in atherosclerotic plaque destabilization and rupture.

Poster Session

RESVERATROL PROTECTS AGAINST ATHEROSCLEROSIS DEVELOPMENT IN *APOE*3-LEIDEN.CETP* MICE

Jimmy Berbée[1], M.-C. Wong[1], Y. Wang[1], J. van der Hoorn[2], H. Romijn[1], L. Havekes[1,2], H. Princen[2], P. Rensen[1]

[1]*Endocrinology and Metabolic Diseases, LUMC,* [2]*TNO-Biosciences, Leiden, The Netherlands*

Introduction: Resveratrol is a natural activator of the deacetylase SIRT1. Activation of SIRT1 has been shown to extend lifespan and improve insulin sensitivity. We assessed whether resveratrol also protects against atherosclerosis development by using *APOE**3-*Leiden*(*E3L*).*CETP* mice, a unique model for human-like lipoprotein metabolism that shows a similar response to lipid-lowering and HDL-raising drugs as humans.

Methods: *E3L.CETP* mice (n=15/group) were fed a Western-type diet containing 0.15% cholesterol for 5 weeks as run-in. Subsequently (t=0), mice were fed this diet with or without supplementation with resveratrol (0.01% w/w). Plasma was collected every 4 weeks for determination of lipids and inflammatory parameters. Atherosclerosis was assessed after 14 weeks in the aortic root.

Results: Resveratrol reduced plasma TC by approx. 10-20% throughout the intervention period reaching statistical significance as compared to control treatment at week 4 and 8 ($P< 0.05$). Resveratrol specifically reduced (V)LDL-C (-21%) and did not affect HDL-C. As a result, resveratrol tended to reduce the total TC exposure by -14% ($P=0.057$). Resveratrol did not reduce systemic inflammation (plasma SAA and a panel of cytokines). Resveratrol markedly reduced the atherosclerotic lesion area by -52% ($P< 0.01$) and induced more stable lesions as indicated by an increased smooth muscle cell area (+50%; $P< 0.05$) and collagen area (+42%; $P< 0.01$), and a decreased macrophage area (-22%; $P=0.057$).

Conclusions: Resveratrol protects against the development of atherosclerosis and induces a more stable lesion phenotype. Therefore, activation of SIRT1 may be a novel strategy to treat CVD, and we are currently investigating the underlying mechanism.

Poster Session

THE ROLE OF EXERCISE AND ENDOCANNABINOID SYSTEM IN ATHEROSCLEROTIC PLAQUE STABILITY - EXPERIMENTAL STUDY

Michalis Katsimpoulas[1], N. Kadoglou[1], A. Kapelouzou[1], P. Moustardas[1], I. Botis[1], E. Dede[1], N. Kostomitsopoulos[1], C. Liapis[2], P. Karayannacos[1]

[1]Center for Experimental Surgery, Biomedical Research Foundation, Academy of Athens, [2]Vascular Surgery, Medical School, University of Athens, Athens, Greece

Objective: To investigate the effects of exercise program and a cannabinnoid receptor antagonist (rimonabant) on atherosclerotic plaque stability in apolipoprotein E-knockout (apoE$^{-/-}$) mice.

Methods: Forty male apoE$^{-/-}$ mice were fed a western-type diet for 16 weeks. Thereafter, mice were randomly assigned to four equivalent (n=10) groups for 6 additional weeks: A) Control (CG): Received normal-diet, B) Exercise (EG): Normal diet+exercise training on a motorized treadmill (5times/week, 60min/session), C) Rimonabant (RG): Normal diet+Rimonabant administration (20mg/kg), D) Combined treatment (ERG): Normal diet+exercise+rimonabant. We determined lesion area and the concentrations of macrophages, collagen, elastin and MMP-9. One-way ANOVA was used for statistical analysis ($p< 0.05$).

Results: Compared to CG (58.21±4.31%), the percentage of aorta lumen stenosis was significantly reduced across groups RG (43.71±5.2%;$p=0.002$), EG (44.13±6.1%;$p< 0.001$) and ERG (34.66±7.7%;$p< 0.001$). Besides this, all active groups showed a considerable downregulation in macrophages and MMP-9 content in their atherosclerotic lesions ($p< 0.05$). Between groups analysis revealed an even greater reduction in macrophages and MMP-9 in the combined-treatment group than RG ($p=0.008$) and EG ($p=0.015$). Similarly, collagen and elastin concentrations increased in either exercise or rimonabant-treated groups than CG ($p< 0.05$), while the combined intervention conferred an additional more than 40% increment ($p< 0.05$). At the end, body-weight and lipid parameters did not differ between groups ($p>0.05$).

Conclusion: Treatment combining exercise plus rimonabant administration exerted better results on atherosclerotic plaque stability in apoE$^{-/-}$ mice, than each intervention alone. Those effects seemed not to be mediated by weight-loss or lipid profile changes.

Poster Session

ATORVASTATIN REDUCES MACROPHAGE ACCUMULATION IN THE ATHEROSCLEROTIC PLAQUE IN PATIENTS UNDERGOING CAROTID ENDARTERECTOMY: A COMPARISON VERSUS NON-STATIN BASED REGIMEN

Massimo Puato[1], F. Elisabetta[1], M. Rattazzi[1], F. Cipollone[2], A. Zambon[3], F. Grego[4], A. Mezzetti[2], P. Pauletto[1]

[1]Dipartimento di Medicina Clinica e Sperimentale, Università di Padova, Padova, [2]Center of Excellence on Atherosclerosis, Hypertension and Dyslipidemia, Università di Chieti-Pescara, Chieti, [3]Dipartimento di Scienze Mediche e Chirurgiche, [4]Dipartimento di Scienze Cardiologiche Toraciche e Vascolari, Università di Padova, Padova, Italy

Objective: To compare the effect of high dose vs low dose of atorvastatin vs non-statin based treatment (cholestyramine plus sistosterol) on cell composition of carotid plaque.

Methods: We recruited 60 hypercholesterolemic patients (total cholesterol, TC, 225-295 mg/dl) eligible for carotid endarterectomy. Three months prior surgery patients were randomized into 3 groups (n=20) receiving atorvastatin 10 mg/day (AT-10), or atorvastatin 80 mg/day (AT-80), or cholestyramine 8 g/day plus sitosterol 2.5 g/day (C-S). Analysis of cell composition was performed on endarterectomy specimens.

Results: The three treatments resulted in a significant reduction of TC and LDL cholesterol (LDL-C), although the decrease in TC and LDL-C was of smaller magnitude in the C-S group. The three regimens did not influence the levels of inflammatory markers (including hs-CRP). The macrophages content was significantly lower in the AT-10 group plaques compared to the C-S group. It was further reduced in the AT-80 group plaques. These differences were still present but no longer significant after adjustment for changes in LDL-C. No difference in lymphocyte number was observed among treatments, while the content of smooth muscle cells was higher in the AT- 80 group. An inverse association was observed between LDL-C changes in the three groups and macrophage content in the plaques.

Conclusions: A short-term treatment with high-dose statin is superior to a non-statin lipid lowering regimen in reducing the macrophage cell content inside atherosclerotic lesions, and this effect is significantly modulated by the degree of LDL-C lowering.

Poster Session

HIGH-THROUGHPUT SERUM NMR METABONOMICS - METABOLIC PHENOTYPING FOR SCREENING AND PROGNOSTICS OF VASCULAR DISEASES AND THEIR COMPLICATIONS

Markku Savolainen[1,2], T. Salonurmi[1], M.-R. Järvelin[2,3,4], T. Tukiainen[2], P. Wurtz[2], A. Kangas[2], P. Soininen[2,5], M. Ala-Korpela[1,2,5]

[1]Dept. of Internal Medicine and Biocenter Oulu, [2]Computational Medicine Research Group, University of Oulu, [3]Dept. of Child and Adolescent Health, National Institute for Health and Welfare, Oulu, Finland, [4]Department of Epidemiology and Public Health, Imperial College, London, UK, [5]NMR Metabonomics Laboratory, University of Kuopio, Kuopio, Finland

Many common diseases and metabolic disturbances are characterised by the development of micro- and macrovascular complications. Early detection of high risk individuals would aid in preventing clinical consequences.

A high-throughput nuclear magnetic resonance (NMR) metabonomics approach is introduced to characterise individual systemic phenotypes. The methodology is based on two molecular windows for native serum - LIPO and LMWM. The LIPO window gives information, for example, on the lipoprotein subclass distribution and the LMWM window on various low-molecular-weight metabolites such as glucose, alanine, and creatinine. In addition, a recently established NMR protocol for serum lipid extracts produces detailed molecular information on serum lipid constituents such as free and esterified cholesterol, phosphatidylcholine, sphingomyelin, and ω-3 fatty acids. The fully automated analysis has a capacity of up to 180 LIPO + LMWM or LIPID windows in 24h. In total, approximately 140 metabolic measures can be quantified from each serum sample.

The new instrumental set-up for NMR metabonomics and an overview of our recent results from serum samples in the Northern Finland Birth Cohort 1966 (n~6,000) are presented. The metabolic phenotypes are discussed with respect to various epidemiological variables, risk assessment, diagnostics, and genome-wide data. We demonstrate the inherent suitability of serum NMR metabonomics to identify subtle changes in lipoprotein subclass-related metabolism as well as in various other metabolites of high interest.

Our work reveals the diffuse genotypic and phenotypic nature of vascular problematics and the limitations of single 'diagnostic' biomarkers. However, it promises cost-effective solutions via high-throughput analytics and advanced computational methods.

Poster Session

EVALUATION OF SYSTEMIC INFLAMMATION IN FAMILIAL HYPERCHOLESTEROLEMIA BY BIOMARKER PROFILING ON BIOCHIP ARRAYS: IMPACT OF LDL-APHERESIS

M.J. Chapman[1,2,3], P. Robillard[1,2,3], A. Orsoni[1,2,3], S. Saheb[4], M. Atassi[4], A. Kontush[1,2,3], J. Lamont[5], E. Bruckert[6]

[1]INSERM U939, [2]Université Pierre et Marie Curie-Paris, [3]Hôpital de la Pitié-Salpétrière, APHP, Paris, France, [4]Haemobiotherapy Unit, [5]Randox Laboratories, Crumlin, Ireland, [6]Department of Endocrinology, Hôpital Pitié-Salpétrière, APHP, Paris, France

Objective: Inflammation is central to the pathophysiology of the premature atherosclerotic disease characteristic of Familial Hypercholesterolemia. Extracorporal removal of atherogenic apoB-containing lipoproteins represents a highly efficacious, acute therapy in severe FH phenotypes. Monitoring of a constellation of circulating levels of inflammatory biomarkers may provide insight into the efficacy of apheresis in individual patients and may therefore impact the choice of add-on therapy.

Methods and results: Biochip arrays incorporating multiple specific antibodies to proteins implicated in inflammation and atherogenesis were used to evaluate the plasma profile of pro- and anti-inflammatory cytokines and of cellular adhesion proteins pre- and post-LDL-apheresis, and compared with levels in normolipidemic, healthy controls. LDL-apheresis significantly reduced levels of ICAM-1, E-selectin, P-selectin and L-selectin (-9 to -43%; $p < 0.01$) to concentrations equal or less than those in controls, indicative of attenuation of the adhesion of platelets and leukocytes to vascular endothelium. Supranormal levels of anti-inflammatory IL-4 were unaffected by LDL-apheresis while those of IL-10 were increased (+33%; $p < 0.01$). By contrast, supranormal levels of pro-inflammatory IL-2 and TNFα were unchanged. Finally apheresis induced significant elevation in levels of pro-inflammatory IL-6 (+63%; $p < 0.001$), IL-8 (+11%; $p < 0.05$) and interferon γ (+55%; $p < 0.05$), potentially as a consequence of surface activation of inflammatory leukocytes during the apheresis procedure.

Conclusion: Biochip profiling allows comprehensive evaluation of the impact of LDL-apheresis on the overall systemic inflammatory balance in FH patients. This procedure reduces circulating protein levels for inflammatory cells but exerts inconsistent effects on the profile of pro- versus anti-inflammatory cytokines.

Poster Session

A NUTRIGENOMIC APPROACH TO STUDY THE EFFECT OF HERBAL POLYPHENOLS ON ATHEROGENICTRANSCRIPTOME

Veena Dhawan[1], N. Malik[1], A. Shukla[1], K. Sikand[1], A. Bahl[2], D. Kaul[1]

[1]Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, UT, India,
[2]Cardiology, Postgraduate Institute of Medical Education & Research, Chandigarh, UT, India

Crosstalk amongst the genes coding for LDLR, LXR alpha, PPARs (alpha, gamma), CD-36, c-myc, COX-2 and MMP-2 play crucial role in atherosclerosis. Certain herbal preparations have been identified to have beneficial effects on cardiovascular pathophysiology. The present study was therefore undertaken to explore the effect of herbal polyphenols on the regulation of genes involved in atherogenesis. Polyphenols were extracted from the leaves of Green tea (GrTP), Neem (NP) and Tulsi (TP) and bark of *Terminalia arjuna (TA)* purified by HPLC. Sub-fractions dialysed against normal saline were used for culture experiments. Normal human PBMCs were cultured in presence of polyphenols from GrTP, NP and TP in different dose (0-120μg/ml). mRNA expression of LDLR, LXRα, PPARs, CD-36 and c-myc genes was determined. In another study, PBMCs from 40 subjects with proven coronary artery disease (Group I) and 40 controls (Group II) were cultured in presence of the extract of *TA* (TAE) (0-150ug/ml).Time and dose-dependent studies were carried out and transcriptional and translational expression of IL-18, MMP-2 and COX-2 were determined. Our data indicated that GrTP (Fr.II) and Tulsi (Fr.IV) significantly downregulated PPAR-Y, CD-36 and LXRα and upregulated mRNA expression of PPARα. A mixed effect was observed on c-myc and LDLR genes. Exposure of PBMCs to TAE revealed a dose-dependent downregulation of IL-18, COX-2 and MMP-2 both at the transcriptional and translational level. We propose that these polyphenols may have crucial phytotherapeutic and anti-inflammatory potential in the treatment/prevention of atherosclerosis. Future studies are warranted to prove their efficacy *in vivo*.

Poster Session

LIPID-LOWERING EFFECT OF METHANOLIC EXTRACT OF VERNONIA AMYGDALINA LEAVES IN RATS FED ON HIGH CHOLESTEROL DIET

Oluwatosin Adaramoye, J. Achem, O. Akintayo, M.A. Fafunso

Biochemistry, University of Ibadan, Ibadan, Nigeria

Objective: In this study, the lipid-lowering effect of methanolic extract of Vernonia amygdalina (VA) leaves in rats fed on high cholesterol diet was studied, and compared with a standard hypolipidemic drug; Questran (Qu).

Methods: The effect of VA on the lipid profile of cholesterol-fed rats was assessed by measuring the levels of total cholesterol, triglyceride, Low-density lipoprotein cholesterol (LDL-c), High-density lipoprotein cholesterol (HDL-c), lipid peroxidation (LPO) and glutathione (GSH) in plasma and liver of the animals.

Results: Administration of cholesterol at a dose of 30-mg/ 0.3 ml for 9 consecutive weeks resulted in a significant increase ($p < 0.05$) in plasma and post mitochondrial fraction (PMF) cholesterol levels by 33% and 55%, respectively. Treatment with extract of VA at doses of 100 and 200 mg/kg caused a dose-dependent reduction in plasma and PMF cholesterol by 20%, 23% and 23%, 29%, respectively. Similar reduction in cholesterol levels was obtained in Qu-treated rats. VA at 200 mg/kg decreased the plasma and PMF LDL-c levels by 23% and 49%, and plasma and PMF triglyceride levels by 29% and 28%, respectively. VA at 100 and 200 mg/kg increased the plasma HDL-c levels of the rats 41% and 59%, respectively. Also, VA at 100 and 200 mg/kg decreased the levels of plasma and PMF LPO by 38%, 42% and 35%, 45%, respectively. In addition, VA augmented the cholesterol-induced decrease in PMF glutathione levels of the rats.

Conclusions: Taken together, these results confirm that VA may serve as a potential therapeutic agent for hyperlipidemia.

Poster Session

EVALUATION OF DIABETIC ANGIOPATHIES BY DIGITAL THERMOGRAPHY

Vasile Anestiadi[1], Z. Anestiadi[2], V. Anestiadis[1]

[1]Center for Pathobiology and Pathology, Academy of Sciences, [2]Chair of Endocrinology, Medical University, Chisinau, Moldova

Objective: To determine the potential value of digital thermography (DT) for the evaluation of macro- and microangiopathies of lower limbs in diabetes mellitus (DM).

Methods: We have studied 250 pts with DM, of which 120 male. 100 pts had DM type I, 150 pts had DM type II, with age varying from 27 to 80 yrs, mean age (mean-SEM) was 56.2-6.3 years. Thermograms were acquired at a constant ambient temperature, in fasting state, using a PC-interfaced thermograph. Analysis included obtaining averaged temperature curves (ATC), building of regions of interest (ROI), ROI histograms, viewing in 7 color tables.

Results: Five main thermogram patterns were found:

1. asymmetric unilateral hypothermia (delta-t = 0.3 grades Celsius) of 1st toe (in 35% of DM I cases);

2. bilateral symmetrical hypothermia of both feet at the level of distal phalanges (in 25% of DM I cases);

3. unilateral hypothermia of foot ("thermo-amputation");

4. unilateral hyperthermia of foot and tibial region;

5. focal hyperthermic regions (diameter from 2 to 4 cm.; indicating potential subsequent ulceration).

ATCs and symmetric left-right ROI comparisons helped distinguishing between macro- and microvascular disease, between neuro- and angiopathy in DM, evaluating therapy effectiveness. DT was most valuable in surgical cases, permitting

(1) to locate the optimum level of amputation and

(2) to detect early the inflammation.

Conclusion: DT results in DM correlate well with pathomorphology, and are influenced by DM type, DM duration, and presence of local inflammation.

Poster Session

PLASMA LIPIDOMIC ANALYSIS OF STABLE AND UNSTABLE CORONARY ARTERY DISEASE

Peter Meikle[1], D. Tsorotes[1], C. Barlow[1], J. Weir[1], G. MacIntosh[1], M. Barber[1], B. Goudey[2], J. Bedo[2], L. Stern[2], A. Kowalczyk[2], I. Haviv[1], A. White[1], A. Dart[3], S. Duffy[3], B. Kingwell[1]

[1]Baker IDI Heart and Diabetes Institute, [2]National ICT Australia (NICTA), University of Melbourne, [3]Cardiovascular Medicine, Alfred Hospital, Melbourne, VIC, Australia

Introduction: Worldwide, 19 million people will die from a sudden coronary event annually as a result of disruption of an atherosclerotic plaque. Currently there is no means to predict plaque instability in coronary artery disease (CAD). Traditional risk factors such as age, sex, smoking status, diabetes, hypertension and standard blood lipids fail to adequately identify plaque instability. Lipids play a pivotal role in the progression of CAD and novel lipid species offer the potential to act as biomarkers for plaque instability.

Methods: Modified ceramide and modified phosphatidylcholine species were shown to distinguish stable and unstable CAD. These newly identified biomarkers were measured together with known plasma lipids, including sphingolipids, acylglycerols, and phospholipids, to establish a plasma lipid profile using electrospray-ionisation tandem mass spectrometry. Plasma lipid profiles were determined for 202 participants (control, n=60, stable CAD, n=61, unstable CAD, n=81).

Results: From a total of 337 lipid species, 165 were different between control and CAD groups ($p< 0.01$) while 40 were different between stable and unstable CAD groups ($p< 0.01$). Multivariate analysis using a statistical machine learning approach combined with recursive feature elimination and multiple cross-validation iterations was applied for the creation of prediction models. Comparison of models with varying number of features showed that models with only 8 lipids were sufficient to provide optimal discrimination between stable and unstable cohorts (AUC=0.75) while 16 lipids were sufficient to discriminate control from CAD patients (AUC=0.94).

Conclusions: Plasma lipid profiles can provide improved discrimination, beyond conventional risk factors, between stable and unstable CAD.

Poster Session

DECREASE OF INSULIN SENSITIVITY FOLLOWS THE DISORDERS - FROM OBESITY, THROUGH PRE-DIABETES, TO NEWLY DIAGNOSED DIABETES

Vesna Dimitrijevic-Sreckovic[1], P. Djordjevic[1], B. Sreckovic[2], S. Popovic[1], D. Gostiljac[1], M. Ilic[1], G. Milic[1], T. Nisic[1], I. Soldatovic[3], G. Markovic[1]

[1]Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [2]Internal Medicine, Clinical Center Bezanijska Kosa, [3]Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia

Aims: To examine insulin sensitivity and secretion in obese, pre-diabetic and diabetic patients..

Material and methods: The study included 479 obese individuals (age over 45, body mass index (BMI) >25 kg/m2) classified into four groups: I-obese (243); II- impaired fasting glucose IFG (106); III- impaired glucose tolerance (IGT) (107); IV- newly diagnosed diabetes mellitus type 2 (DM2).

Results: Blood pressure, HbA1C and HOMA IR values followed the progression of disorders: HOMA IR - I-5.5±6.75, II-5.67±3.4, III-6.59±8.4, IV-11.6±4.0 mmol/μU/ml. Highest insulin secretion was found in Groups I and II: HOMA β: I-106.7±105.1, II-68.6±63.7, III-94.1±106.1, IV-32.1±8.6; mean insulin: I-83.2±44.8, II-45.4±44.8, III-46.3±34.6, IV-26.4±4.8. Correlations: WC (p< 0.0l) with HOMA IR and HOMA β; HOMA IR (p< 0.0l) with HOMA β and HbA1C; BMI (p< 0.0l) with WC, HOMA β; glycemia 0 min (p< 0.0l) with HbA1C and HOMA IR; glycemia 30 min with HOMA IR (p< 0.05), HOMA β and HbA1C (p< 0.0l); glycemia 120 min with HOMA IR (p< 0.05), I with HbA1C (p< 0.0l); insulin 0, 30, 120min and mean insulin (p< 0.0l) with WC, HOMA IR I HOMA β.

Conclusion: Decreased insulin sensitivity exists in obesity, decreases in pre-diabetes and is lowest in DM2. Insulin secretion is highest in obesity and IGT and decreases in DM2. Correlations of insulinemia with WC, HOMA IR and HOMAβ confirm the importance of abdominal obesity in disorder etiopathogenesis. High importance of correlation of 0, 30 and 120 min glycemia with HbA1C and HOMA IR confirms the IR connection with the degree of glycoregulation disorder.

Poster Session

GENERATION OF RECOMBINANT ANTIBODIES TO NITROTYROSINE TO FACILITATE THE IDENTIFICATION OF NOVEL PROTEIN BIOMARKERS OF ATHEROSCLEROSIS

Danielle Hof, S. Decasper, A. von Eckardstein

University Hospital Zurich, Zurich, Switzerland

Today, several risk factors, like high blood pressure and smoking, help to identify individuals that are at high risk of developing coronary artery disease. However, there is still a high demand for the identification of novel biomarkers to improve the early diagnosis of acute coronary events and to identify pre-symptomatic patients at high risk for coronary events. This project aims at the identification of such biomarkers. Inflammatory processes play a pivotal role in the progression of atherosclerotic plaques, and we hypothesize that proteins in the plaque are modified during plaque rupture and erosion, and are released into the blood stream. Possible posttranslational modifications include proteolytic cleavage and oxidation of amino acids, such as the formation of nitrotyrosine.

Antibodies against nitrotyrosine - in a protein context - are being isolated from large antibody phage display libraries. So far, we have isolated several different antibody specificities, but recognition of the modified amino acids depended on the composition of the surrounding amino acids. Using affinity maturation by error prone PCR of the recombinant antibody gene, followed by novel selections, we have improved the specificities and affinities of these recombinant antibodies.

Now, we will use these anti-nitrotyrosine antibodies to detect antigens that contain this modified amino acid and that are specifically present in plasma samples from patients with atherosclerotic cardiovascular disease and acute coronary syndromes. Potential targets will be isolated, using the antibodies as baits, and identified by mass spectrometry.

Poster Session

ASSESSMENT OF SUBCLINICAL CORONARY ATHEROSCLEROSIS IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA WITH MULTISLICE COMPUTED TOMOGRAPHY

Miquel Franco[1], F. Carreras[2], R. Leta[3], A. Duran[3], G. Pons-Lladó[3]

[1]UAB, [2]Cardiology, Universitat Autonoma de Barcelona, [3]Cardiology, UAB, Barcelona, Spain

Background: Heterozygous Familial Hypercholeterolemia (HFH) present with early coronary disease, therefore, a premature diagnosis of cardiac involvement would be desirable. Diagnostic methods applied in assymptomatic HFH patients are mainly focused on laboratory parameters, a diagnostic technique suitable for the non invasive coronary artery disease assessment would be desirable.

Objective and method: To compare the prevalence of subclinical coronary atherosclerosis, in a group of patients with HFH in relation to a control group of assymptomatic normal population, using non invasive coronary angiography by multislice computed tomography (CTA) as the diagnostic technique.

Patients. Sixty consecutive asymptomatic patients from the Clinical Lipid Unit of our Hospital were screened, as well as a control group matched to the gender, age and the other risk factors.

Results: Age: 48±14 y.o. Male 34 p. (57 %). BMI 26,5. Risk factors: smokers 45 %, hypertension 6,7 %, diabetes 1,7 %. CTA findings (cases/controls) were: no lesions in 25/35 individuals (p NS), non significant stenosis in 21/18 (p NS), significant stenosis in 13/4 ($p < 0.01$), non conclusive study in 1/3 (p NS). Coronary wall calcification assessed by the Agatston score showed a mean score of 260 in HFH cases, and 48 in controls (p=0,008). No differences were observed in the lipid profile and treatment between patients with significant stenotic lesions in relation to the patients without lesions or with non significant coronary stenotic lesions.

Conclusion: CTA is a useful diagnostic technique to assess the burden of subclinical coronary artery disease in HFH patients, unsuspected by laboratory parameters.

Poster Session

CAROTID ATHEROSCLEROSIS OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA NO LONGER DIFFERS FROM UNAFFECTED RELATIVES ALREADY WITHIN 18 MONTHS AFTER IDENTIFICATION

Roeland Huijgen[1], B.A. Hutten[2], I. Kindt[3], E. de Groot[1], J.J.P. Kastelein[1], M.N. Vissers[1]

[1]Vascular Medicine, [2]Clinical Epidemiology Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, [3]Foundation for the Identification of Persons with Inherited Hypercholesterolemia, Amsterdam, The Netherlands

Background and objective: Familial hypercholesterolemia (FH) is associated with increased premature cardiovascular disease. The FH population has been used as a successful model for studies into atherogenesis, in order to evaluate efficacy of novel treatment regimens. However, the value of FH as a model has been questioned since the recent ENHANCE-trial. It is possible that atherosclerosis of these FH patients, as assessed by carotid intima-media thickness (cIMT) in the ENHANCE-trial, could not be reduced any further due to long-term statin exposure prior to study initiation. To explore this, we compared the cIMT between recently diagnosed FH patients and unaffected relatives.

Methods: We aimed to recruit 300 subjects (18-55 years) who had been tested by the Dutch genetic cascade screening program: 150 FH mutation carriers with LDL-C>P90 and 150 unaffected relatives, all treatment-naïve at screening. A cIMT was performed within 18 months after screening. Differences in cIMT between groups were determined by multivariate models.

Preliminary results: Hitherto, we included 140 FH patients and 135 unaffected relatives. FH patients had higher mean LDL-C levels than the relatives at time of genetic screening (4.6 vs 3.2 mmol/L, respectively; $p< 0.001$). At follow-up, 70% of the patients had initiated cholesterol-lowering medication compared to 10% of the relatives. Median treatment duration was 7 (IQR: 6-15) months. Mean cIMT did no longer differ between the groups (0.66 vs. 0.65 mm, respectively; $p=0.5$).

Conclusion: Our data suggest that carotid intima-media thickening in FH patients can be rapidly restored to normal dimensions, as seen in unaffected relatives.

Poster Session

CHARACTERIZATION OF PATIENTS WITH VERY HIGH PLASMA HDL-CHOLESTEROL, WITH AND WITHOUT VASCULAR PATHOLOGY

Gautamn Sarwal[1,2], A. Al-Sarraf[1], C. Taraboanta[1], J. Frohlich[1,3]

[1]Healthy Heart Prevention Clinic, Providence Heart+Lung Institute, Vancouver, BC, Canada, [2]Mayne Medical School, University of Queensland, Brisbane, QLD, Australia, [3]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Background: While elevated levels of HDL-C have been accepted as antiatherogenic, very high levels of HDL-C may not be atheroprotective as previously described. This suggests that there is a tendency to underestimate a patients' cardiovascular risk when very high HDL-C are present. We carried out a clinical study as opposed to a traditional epidemiological approach by phenotyping patients, at a lipid referral clinic within a major metropolitan hospital, with HDL-C levels $\geq$ 90th percentile.

Objective: To characterize patients with extremely high plasma levels of HDL-C.

Methods and results: Charts of 113 patients with HDL-C $\geq$ 90th percentile cutoffs based on age and gender, from the Healthy Heart Prevention Clinic at St. Paul's Hospital in Vancouver, were reviewed. We extensively phenotyped these patients and found that most lead a healthy lifestyle (low incidence of smoking, physical inactivity, moderate alcohol intake, infrequent obesity and a healthy diet). There was a surprisingly low incidence of Type 2 Diabetes Mellitus - in only two of 113 patients. Despite high levels of HDL-C, the Framingham risk score showed a spectrum of values among the patients instead of indicating an overall low CVD risk. Moreover, 13 were previously diagnosed with CVD, one with a positive cardiac stress test for ischemia and eight with positive ultrasound findings for carotid plaque.

Conclusion: As observed in this clinical cohort, high plasma levels of HDL-C do not irrefutably protect against the onset of CVD.

Poster Session

MODERATE-SEVERE BLEEDING COMPLICATIONS AND CLINICAL OUTCOMES IN PATIENTS WITH ACUTE CORONARY SYNDROME; AN ANALYSIS FROM SIX MIDDLE EASTERN COUNTRIES

Douraid Shakir[1], M. Al Mallah[2], M. Zubaid[3], W. Al Mahamed[4], A. A. Alsheikh-Ali[5], J. Al Suwaidid[1]

[1]Hamad Medical Corporation, Doha, Qatar, [2]Department of Internal Medicine, Henry Ford Health System and Wayne State University School of Medicine, Detroit, IL, USA, [3]HAMAD Medical Corporation Al Kabeer Hospital, Kuwait, Kuwait, [4]Sheisk Khalifa Medical City, Dubai, [5]Sheisk Khalifa Medical City, Abu Dhabi, United Arab Emirates

Background: Little is known about the prevalence and prognostic implications of major bleeding complications among patients admitted with acute coronary syndromes (ACS) in the Middle East. The aim is to study the prevalence and outcome of patients with acute coronary syndrome (ACS) who developed major bleeding complications in Middle Eastern patients.

Methods: The Gulf Registry of Acute Coronary Events (Gulf RACE) is a prospective, multi-national registry conducted in 2007, for a 6-month period for all patients hospitalized with final diagnosis of ACS in 65 centers in 6 adjacent Middle Eastern countries. There were no exclusion criteria. 8104 patients were stratified according to the development of major bleeding complications during the index admission.

Results: Patients with bleeding complications when compared to those without bleeding complications were significantly were significantly older, more likely to present with atypical chest pain and have ST-elevation myocardial infarction. However, there were no significant differences between the two groups in regards to gender or the presence of other cardiovascular risk factors. Patients with bleeding complications had worse in-hospital outcomes. After adjusting for baseline characteristics, major bleeding complication was independently associated with more than a 3-fold increase in in-hospital mortality (OR 3.4, 1.4-8.1, p=0.0063).

Conclusion: Similar to Western studies, bleeding in the setting of ACS is a powerful and independent predictor of poor in-hospital outcomes in patients admitted with ACS in the Middle East.

Poster Session

N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE BUT NOT HIGH-SENSITIVITY C-REACTIVE PROTEIN IS RELATED TO SEVERITY OF CORONARY STENOSIS IN ACUTE CORONARY SYNDROME

Shijun Li, Z. Sun, J. Wang, T. Yang

Chinese PLA General Hospital, Beijing, China

Biochemical indicators of cardiac performance (e.g., B-type natriuretic peptide (BNP) and its N-terminal fragment and inflammation (e.g., high-sensitivity C-reactive protein (hsCRP)) predict mortality in acute coronary syndrome. However, little is known about the relationship of these factors with severity of coronary artery stenosis in patients with acute coronary syndrome.

Methods: 381 subjects including 246 unstable angina pectoris patients, 85 myocardial infarction patients and 50 controls were recruited and classified into three groups: normal-vessel group (50 cases), single-vessel disease group (1- vessels disease) (93 cases) and multiple-vessel disease group (≥2- vessels disease) (238 cases). A selective coronary angiography was performed. Nt-proBNP, hsCRP and blood-lipid levels were measured following hospital admission, and we still elevated relationship of Gensini score of coronary stenosis with Nt-proBNP, hs-CRP, age, sex and blood-lipid level.

Results: There was higher Nt-proBNP level but not hsCRP in patients with myocardial infarction than in patients with unstable angina pectoris. The patients with multiple-vessel disease had significantly high Nt-proBNP level but not hsCRP compared with those with single-vessel disease. Gensini score of coronary stenosis severity was significantly higher in the patients with multiple-vessel disease or myocardial infarction than in those with single-vessel disease or unstable angina pectoris. Further by bivariate correlation and multivariable stepwise regression analysis, we demonstrated that Nt-proBNP but not hsCRP level was related to Gensin score of severity of coronary artery stenosis in acute coronary syndrome.

Conclusion: Nt-proBNP but not hsCRP level is related to severity of coronary artery stenosis in patients in acute coronary syndrome.

Poster Session

SYSTEMIC HYPOXIA PROMOTES THROMBIN GENERATION BY ENHANCING RELEASE OF MONOCYTE-DERIVED MICROPARTICLES DURING EXERCISE

Jang-Shyan Wang

Graduate Institute of Rehabilitation Science, Chang Gung University, Tao-Yuan, Taiwan R.O.C.

Thrombin activity is critical to determining the severity of haemostatic reactions. Oxidative-low density lipoprotein (ox-LDL) promotes the shedding of procoagulant-rich microparticles from monocytes, thereby accelerating the pathogenesis of atherothrombosis. This study explicates the manner in which hypoxic exercise affects monocyte-derived microparticles (MDMP) release and MDMP-mediated thrombin generation (TG) under atherogenetic and inflammatory status. Fifteen sedentary healthy men performed a hypoxic exercise test (50%VO_{2max} for 30 min under 12%O_2 in air) on a bicycle ergometer. At rest and immediately after exercise, the MDMP characteristics and dynamic TG parameters were measured by two-color flow cytometry and calibrated, automatic thrombinography, respectively. The results showed that both ox-LDL and lipopolysaccharide (LPS) increased the levels of total MDMP and the tissue factor-rich and phosphatidylserine-exposed MDMP, which were accompanied by elevated the height of thrombin peak and increased the rate of TG in the MDMP-rich plasma (MRP). Hypoxic exercise increased total and procoagulant-rich MDMP counts and accelerated TG in plasma, whereas MDMP-promoted peak height and rate of TG under both ox-LDL and LPS stimulations also enhanced in response to this exercise regimen. Therefore, we conclude that hypoxic exercise promotes atherogenetic or inflammatory factor-induced TG by increasing the release of procoagulant-rich microparticles from monocytes.

Poster Session

ASSOCIATION OF ET-1, MMP-9, AND MYELOPEROXIDASE WITH CORONARY MORPHOLOGY DETECTED BY CT ANGIOGRAPHY IN MODERATE - HIGH RISK ASYMPTOMATIC SUBJECTS

Yishai Ben Moshe[1], E. Lubovich[2], Y. Rivlin[2], I. Maor[3], R. Zeina[4], U. Rosenschein[2], E. Goldhammer[1]

[1]Cardiac Rehabilitation, [2]Cardiology, [3]Biochemistry, [4]Radiology, Bnai Zion Medical Center, Haifa, Israel

Objectives: To study possible association between **serum ET-1**, **MMP- 9**, and **MPO** and coronary artery morphology in cardiac CT angiography (CTA) in intermediate and high risk asymptomatic subjects.

Background: CTA has been established as an acceptable method for the diagnosis of coronary artery disease (CAD) in symptomatic low risk, and in intermediate - high risk asymptomatic subjects. Biomarkers like ET-1, MPO, and MMP-9 were found to be involved in the atherosclerotic plaque development and in various clinical manifestations of CAD.

Methods: 65 asymptomatic subjects referred for the evaluation of CAD by CTA were included. Obstructive CAD was defined when a ≥50 % stenosis of one or more coronary segments was found. Plaques associated with CT Hounsfield attenuation number of ≥ 130 were defined calcified, and 121 or less were as soft.

Results: 666 diseased segments were found, 8.6% were significant, ≥50%, and 91.4% were mild ≤50% lesions, 76 lesions were soft plaques, 11 calcified, and 251 combined, calcified and soft plaques. ET-1 was significantly elevated in subjects with significant obstructive disease (p=0.022), and correlated with calcified plaque burden (r = 0.86, p=0.006). ET-1 was an independent determinant of lesion severity (R^2 = 0.124**,** p= 0.008**).** MMP-9 and MPO did´t correlate with the presence of calcified or soft plaques or with number of diseased segments.

Conclusions: ET-1 may be a marker of significant CAD and calcified plaque burden in asymptomatic subjects. The significance of MMP-9 and MPO as markers of atherosclerosis in such subjects has not been definitely clarified.

Poster Session

THE ANTI-INFLAMMATORY EFFECTS OF EXERCISE TRAINING PROMOTE ATHEROSCLEROTIC PLAQUE STABILIZATION IN APOE-KNOCKOUT MICE WITH DIABETIC ATHEROSCLEROSIS

Nikolaos Kadoglou[1], A. Kapelouzou[1], P. Moustardas[1], M. Katsimpoulas[1], A. Giagini[1], E. Dede[1], N. Kostomitsopoulos[1], C. Liapis[2], P. Karayannakos[1]

[1]Center of Experimental Surgery, Biomedical Research Foundation, Academy of Athens, [2]Department of Vascular Surgery, Medical School, University of Athens, Athens, Greece

Objectives: The present study investigated whether exercise training may affect atherosclerotic plaque development and composition via inflammatory-related pathways in apolipoprotein E knockout ($apoE^{-/-}$) mice with diabetic atherosclerosis.

Methods: A total of 45 $apoE^{-/-}$ female mice (aged 8 weeks) were randomized to the following equivalent (n=15) groups: A)Control, B)Sedentary, C)Exercise. High-fat, diabetogenic diet was administered to all groups for 16 weeks. Subsequently, control mice were euthanatized, while the sedentary and exercise group were placed on normal-diet for 6 additional weeks. Exercising mice followed a program on a motorized-treadmill (60min/session, 5-times/week). At the end of the study, morphometry and characteristics of atherosclerotic plaque stability were assessed in aortic sinus by immunohistochemistry and glucose tolerance test (GTT) was performed.

Results: A considerable ($p< 0.001$) regression in atherosclerotic lesions was observed in the exercise-treated group compared to control and sedentary groups, despite diabetes induction (impaired GTT). We found macrophages, matrix metalloproteinase-3 (MMP-3), MMP-8, MMP-9 and IL-6 concentrations to be considerably ($p< 0.05$) attenuated within the atherosclerotic plaques of the exercise group. Compared to both control and sedentary groups, exercise training significantly increased collagen ($p=0.028$, $p=0.011$, respectively), elastin ($p=0.032$, $p=0.029$, respectively), and TIMP-2 ($p=0.041$, $p=0.032$, respectively) content in the atherosclerotic plaques. The above effects occurred without significant changes in body-weight, lipids or glycemic control. Notably, plasma MMP-3 and MMP-9 followed similar trends as their atherosclerotic tissue concentrations.

Conclusion: Exercise training reduced and promoted atherosclerotic lesions stability in $apoE^{-/-}$ mice with diabetic atherosclerosis. Those effects might be mediated by a favorable modification of inflammatory regulators.

Poster Session

ELEVATED ADVANCED GLYCOSYLATION ENDPRODUCTS (AGE) IN NON-DIABETIC SUBJECTS WITH ATHEROSCLEROSIS

SA Bovenberg[1], A. Westzaan[1], S. Martinez-Hervas[2], G.J. van de Geijn[3], H. Janssen[3], T.L. Njo[3], R. van Mechelen[4], A. Smit[5], J.W.F. Elte[1], M. Castro Cabezas[1]

[1]Intern Medicin, Sint Franciscus Gasthuis, Rotterdam, The Netherlands, [2]Endocrinology, Hospital Clínico Universitario de Valencia, Valencia, Spain, [3]Clinical Chemistry, [4]Cardiology, Sint Franciscus Gasthuis, [5]Intern Medicin, Academic Medical Center, Groningen, Rotterdam, The Netherlands

Background: Advanced glycation endproducts (AGE) have been postulated to play a role in the generation of atherosclerosis in diabetes, but may also play a role in non-diabetic subjects. AGEs can be measured non-invasively using their fluorescence characteristics in the skin. AGEs measured by skin autofluorescence (AF) are both carbohydrate- and lipid-derived, so skin AF is a non-invasive marker for both glycation and oxidative stress.

Aim: To investigate whether AGEs can differentiate between subjects with and without coronary sclerosis but without diabetes, using skin auto-fluorescence.

Methods: Clinical data and skin auto-fluorescence were obtained in non-diabetic patients (n=22) with coronary sclerosis (established by coronary angiography or with a history of cardiovascular disease) and in healthy volunteers (n=74).

Results: There were no differences between patients with and without atherosclerosis with respect to glucose. AGEs were increased in patients with coronary sclerosis (2,63 au ± 0.62) compared to healthy volunteers (2.01 au ± 0.46; P< 0.001). Correlation analysis (Spearman's rho [r]) showed that AGEs were associated with age (r= 0.62 P< 0.01), kreatinin (r= 0.37; P< 0.01), glucose (r= 0.22; P< 0.05) and triglycerides (r= 0.23; P< 0.05). Logistic regression analysis, corrected for age, triglycerides and glucose, showed that AGEs correlated significantly with the presence of coronary sclerosis (odds ratio: 3.87; CI: 1.10-13.64, P< 0.05).

Conclusion: These data show that skin auto-fluorescence is elevated in non-diabetic subjects with coronary sclerosis, similarly to the situation in T2DM.

Poster Session

OXLDL/BETA2GPI COMPLEX AS AN EARLY MARKER OF ATHEROSCLEROSIS INITIATION IN HEALTHY MIDDLE-AGED MEN WITH ABDOMINAL OBESITY

Pavel J. Kraml[1], P. Syrovátka[2], J. Potočková[1], M. Anděl[1]

[1]2nd Dept. of Internal Medicine, 3rd Faculty of Medicine, Charles University, [2]Clinic of Cardiology, Institut of Clinical and Experimental Medicine, Prague, Czech Republic

Rationale: Plasma concentrations of oxLDL/beta2GPI complex are traditionally associated with autoimmune diseases, e.g. SLE, antiphospholid syndrome. Recently, its increased concentrations have been observed in patients with T2DM, cardiovascular diseases (CVD, PVD) and renal failure. It has been proposed that oxLDL/beta2GPI complex represents a link between oxidative stress, inflammation and atherosclerosis.

Objective: To determine whether oxLDL/beta2GPI plasma levels reflect insulin resistance, inflammation and markers of endothelial damage in middle-aged men of primary prevention with abdominal obesity.

Methods: Using the IDF metabolic syndrom criteria (waist circumference > 94 cm), 72 healthy primary prevention men were divided into obese and non-obese subgroups (41 obese men, mean age 51.5+-7.80 years vs. 31 non-obese men; mean age 41.6+-9.37 years; $p>0.05$). All subjects underwent general physical examination, anthropometric measurement, carotid sonography and venous blood was taken for basic biochemical tests, plasma lipids, immunokines and markers of inflammation, adipocytokines, markers of oxidative stress and endothelial dysfunction.

Results: When compared with controls, the subgroup of obese men had significantly higher values of oxLDL/beta2GPI, oxLDL and AOPP (all $p< 0.01$), however in regression analysis only oxLDL/beta2GPI correlated with the immunokine- IL-8 and endothelial dysfunction marker-PAI-I (both $p< 0.01$). Multiple regression analysis revealed that the correlation of oxLDL/beta2GPI with PAI-I remained significant independently of triglycerides, BMI and WHR.

Conclusion: In early-stage of insulin resistance, plasma concentration of the oxLDL/beta2GPI complex reflects both inflammation and endothelial dysfunction.

Increased oxLDL/beta2GPI may thus serve as a marker of atherosclerosis initiation on the endothelium level.

Supported by MSM0021620814.

Poster Session

AN ERYTHROPOIETIN RECEPTOR (EPOR) IN HUMAN ADIPOSE TISSUE (AT)

Kristina Möller[1], E. Piella[2], P. Algenstaedt[2], D.E. Müller-Wiefel[1]

[1]Pediatric Nephrology, Children`s Hospital of the Universitiy of Hamburg, [2]Endocrinology, Medical Clinic of the Universitiy of Hamburg, Hamburg, Germany

AT is an endocrine organ - secreting inflammatoric and antiinflammatoric adipocytokines playing an important role in the development of atherosclerosis. However, few is known about the regulation of the expression of adipocyte derived products. The observation that renal patients under therapy with an erythropoietin stimulation factor (ESF) have higher serum-adiponectine concentrations than those without therapy leads to the hypothesis that there might be a tissue specific EPOR in AT. We investigated the expression of an EPOR in human AT under the influence of ESF. AT of patients was harvested during an elective surgery. Probes were incubated with ESF vs simple culture medium. The m-RNA of the adipocytes was isolated, a relative quantification by real-time PCR and the sequencing of all three domains of the EPOR was performed. The EPOR and the phosphorylation of Stat5 and Jak2 on protein level were examined by Western blot technique. The expression of an EPOR on RNA and protein level could be demonstrated in AT. After stimulation with ESF there was an increase of mRNA-expression of the EPOR and an increase of the expression of phosphorylated Jak2 and Stat5. Data show for the first time an expression of an EPOR in human AT and its regulation by ESF. Therefore a direct influence of ESF on the regulation of the expression of adipocytokines in human AT can be assumed. The EPOR could be an important factor in the regulation of the endocrine functions of AT and might play a protective role in the development of atherosclerosis.

Poster Session

VARIANT IN KIF6 PREDICTS CORONARY EVENTS AND EVENT REDUCTION FROM STATIN THERAPY: CARE, WOSCOPS, PROSPER AND PROVE IT-TIMI 22 STUDIES

Olga Iakoubova

Cardiovascular Diseases, Celera, Alameda, CA, USA

Aims: To identify and validate genetic variants associated with risk for coronary events and differential response to statin therapy, we investigate the association between a 719Arg variant of kinesin family member 6 (encoded by the gene *KIF6*) and coronary events in placebo groups of CARE and WOSCOPS and response to statin therapy in the CARE, WOSCOPS, PROSPER and PROVE IT-TIMI 22 trials.

Results: In placebo-treated patients, carriers of the *KIF6* 719Arg allele (59% of population) had a hazard ratio of 1.50 (95%CI 1.05 - 2.15) in CARE, an odds ratio of 1.55 (95%CI 1.14 - 2.09) in WOSCOPS, and a hazard ratio of 1.28 (95%CI 0.98 - 1.69) in PROSPER patients with prior vascular disease. Among 719Arg carriers, the absolute risk reduction from pravastatin therapy was 4.9% (95%CI 1.8 - 8.0) in CARE, 5.5% (95%CI 3.5 - 7.5) in WOSCOPS, and 6.3% (95%CI 2.5% - 10.0%) in PROSPER patients with prior vascular disease. In contrast, no significant risk reduction was observed among noncarriers in any of these studies. In PROVE IT-TIMI 22, benefit from high-dose, compared with standard-dose, statin therapy was significantly greater in carriers (p=0.018 for interaction between 719Arg carrier status and treatment) with an absolute risk reduction of 10.0% (95%CI 4.9% - 15.0%) in carriers versus 0.8% in noncarriers.

In conclusion, we have idenified a *KIF6* 719Arg variant that predicts risk of coronary events and event reduction during pravastatin therapy. In addition, carriers also received significantly greater benefit (event reduction) from high-dose statin therapy than noncarriers received.

Poster Session

HOMOCYSTEINE AS A CARDIOVASCULAR RISK MARKER IN POLYCYSTIC OVARY SYNDROME

Aleksandra Atanasova[1], G. Dimitrov[1], S. Biljali[2]

[1]University Clinic for Gynecology and Obstetric, [2]Institute for Clinical Chemistry, Skopje, FYR Macedonia

Polycystic ovary syndrome (PCOS) is a condition characterized by oligomenorhea and androgen excess, affects 6-10% of the women in reproductive period. Although the pathogenesis is still uncertain, many studies suggest that PCOS may increase risk for several conditions including insulin resistance, type 2 diabetes, dyslipidemia, hypertension, cardiovascular risk. Woman with PCOS would be expected to be at significantly increased risk for atherosclerotic disease. Hyperhomocysteinemia has been shown as independent predictor of cardiovascular mortality in patients with atherosclerosis. The aim of our study was to determinate levels of homocysteine in woman with polycystic ovary syndrome compared with healthy woman. Thirty two patients (age, 23, 5 ± 5.5) with PCOS and twenty five (age, 25.5± 4.3) healthy woman were involved in the study. Blood samples were collected in early follicular phase. Total homocysteine was measured using fluorescent immunoassay. Statistically significant differences in serum concentration of homocysteine were observed between groups. Mean homocysteine level we found as (10.2± 2.9 vs. 7.0±1.5) in PCOS and normal group respectively ($p< 0.05$). For Macedonian population we found statistically significant increased homocysteine levels in woman with PCOS. Although the mean homocysteine levels are within normal limits, there are significant higher mean homocysteine concentrations between these two groups. Because increased concentrations of tHcy has been shown as an independent risk factor for cardiovascular alterations, it is essential that in this group of woman are taken measures for early prevention.

Poster Session

INFLUENCE OF ARTERIAL STIFFNESS, BODY CONSISTENCY AND SECONDHAND SMOKE EXPOSURE ON CHRONIC VALVE DISEASE AND MYOCARDIAL INFARCTION

Adam Domonkos Tarnoki[1], D.L. Tarnoki[1], Z. Garami[2], G. Veress[3]

[1]Department of Radiology and Oncotherapy, Semmelweis University, Budapest, Hungary, [2]Methodist Hospital DeBakey Heart and Vascular Center, Houston, TX, USA, [3]State Hospital of Cardiology, Balatonfured, Hungary

Objective: Pulse Wave Velocity (PWVao) and Augmentation Index (AIxbra) are associated with the structural changes of arteriosclerosis but never investigated between the two subject groups.

Aim: To determine the arterial stiffness, body consistency and secondhand smoke (SHS) exposure among patients suffering from chronic valve disease (CVD) and myocardial infarction (MI).

Methods: 138 patients were examined (97 MI and 41 CVD) by TensioMed Arteriograph, OMRON BF500 body consistency monitor (via bioimpendance analysis) and a detailed questionnaire.

Results: Higher Aixbra was found in MI group (0,85±25,5 vs. -2,9±31%). PWVao was higher in CVD group (12±3,2 vs. 11,2±2,5 m/s). PWVao and Aixbra worsen by aging in both groups. MI group had higher peripherial blood pressure (138/77 vs. 132/74 Hgmm), SBPao (137,7±22,5 vs. 130,3±19,3 Hgmm), MAP (97,6±11,6 vs. 93,19±9,2 Hgmm), decreased DRA (39,64±11,2 vs. 43,87±15,8) and DAI (46,4±9,7 vs. 52±7%), respectively. Higher BMI (29,19±4,6 vs. 27,54±3,7 kg/m^2), body fat% (32,49±10,8 vs. 30,68±10,2%), visceral fat (12,3±4,3 vs. 10,41±3,9), smoking rate (52,6 vs. 46,3%) and duration (26,6 vs. 22,3 years), significant higher home, workplace and other SHS exposure (1,27 vs 0,45, 1,47 vs 1,03, 0,45 vs 0,27 hours/day) were present in MI group.

Conclusions: Increased and abnormal arterial stiffnesses were found in both groups indicating presence of atherosclerosis. Adverse body consistency (higher body fat% and visceral fat) and higher SHS exposure indicate an increased risk of MI.

Poster Session

CARDIOPROTECTIVE EFFECT OF RED WINE

Aleksandra Novakovic[1], L. Gojkovic Bukarica[2], D. Nezic[3], M. Peric[3], V. Kanjuh[4]

[1]Dept. of Pharmacology, Faculty of Pharmacy, [2]Dept. of Pharmacology, School of Medicine, [3]Institute of Cardiovascular Diseases "Dedinje", [4]Academy of Sciences and Arts, Belgrade, Serbia

The lower incidence of coronary artery disease in the Soutern French and other Mediterranean populations, despite a diet rich in saturated fat and high smoking habits (the so-called French paradox), has been atributed to the prolonged and moderate red wine consumption by these population. It is considered that resveratrol, as a polyphenol, is presented in red wine in significant amounts, and partly responsible for cardiovascular benefits associated with wine consumption. The mechanism of cardiovascular benefits probably includes vasorelaxation, antioxidant and anti-platelet effects of resveratrol. The mechanisms by which resveratrol causes vasodilatation are uncertain.

Aim: The aim of this study was to investigate the mechanism (s) of resveratrol-induced vasorelaxation in human internal mammary artery (HIMA) with endothelium and without endothelium.

Methods: HIMA were precontracted with phenylephrine (10 mM).

Results: Resveratrol induced a concentration-dependent relaxation of the rings with endothelium and without endothelium. Highly selective blocker of ATP-sensitive K^+ channels, glibenclamide as well as nonselective blockers of Ca-sensitive K^+ channels, tetraethylammonium did not block resveratrol-induced relaxation of HIMA rings. Charybdotoxin, a blocker of calcium-sensitive K^+ channels did not affect the resveratrol-induced relaxation. 4-Aminopyridine, non selective blocker of voltage-gated K^+ (K_V) channels, and margatoxin that inhibits K_V1 channels abolished relaxation of HIMA rings induced by resveratrol.

Conclusions: In conclusion, we have shown that resveratrol can induce relaxation of HIMA with endothelium and without endothelium It seems that 4-aminopyridine- and margatoxin-sensitive K^+ channels located in vascular smooth muscle mediated relaxation of HIMA produced by resveratrol.

Poster Session

MATRIX METALLOPROTEINASE-9 (MMP-9), ITS TISSUE INHIBITOR TIMP-1 AND NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AS NEW ATHEROSCLEROSIS MARKERS IN CHRONICALLY DIALYZED PATIENTS

Kinga Musial, D. Zwolinska

Department of Pediatric Nephrology, Wroclaw Medical University, Wroclaw, Poland

Background: Patients on chronic dialysis are prone to cardiovascular complications, being the first cause of morbidity and mortality. The MMP/TIMP proteolytic system may play an essential role in atherosclerosis, but the data concerning such influence in dialyzed patients are scarce.

The aim of study was to assess concentrations of MMP-9 (gelatinase B) with anti-atherogenic activity, its specific inhibitor TIMP-1 and NGAL, preventing MMP-9 degradation by forming a dimer, in children and young adults on automated peritoneal dialysis (APD) and on hemodialysis (HD).

Material and methods: 22 children on APD, 17 patients on HD and 23 controls were examined. Serum concentrations of MMP-9, TIMP-1 and NGAL were assessed by ELISA. Serum CHOL, HDL-CHOL, LDL-CHOL, TGL and hsCRP were also evaluated.

Results: Median values of MMP-9, TIMP-1, NGAL and MMP-9/NGAL ratio were significantly elevated in all dialyzed children vs. controls and were higher in HD than in APD. TIMP-1 values in HD exceeded 3 times those in APD. Consequently, the MMP-9/TIMP-1 ratio, although increased in APD ($p< 0.00001$) and HD ($p< 0.01$), was higher in APD than in HD ($p< 0.00001$). MMP-9 correlated with TGL in HD, TIMP-1 - with HDL-CHOL in HD and with hsCRP in APD. NGAL correlated with CHOL, HDL-CHOL and hsCRP in APD.

Conclusions: Our results suggest the dysfunction of MMP/TIMP system in children on dialysis, with APD being less atherogenic than HD. Correlations with lipid profile and hsCRP may suggest the role of MMP-9, TIMP-1 and NGAL as new markers of atherosclerosis in the examined population.

Poster Session

THE TREATMENT OF COLESTERYL STORAGE DISEASE (CESD) BY EZETIMIBE MONOTHERAPY

Francesca Abello[1], O. Guardamagna[1], V. Baracco[1], R. Bonardi[2]

[1]Department of Pediatrics, [2]Department of Gastroenterology, University of Turin, Turin, Italy

Objective: CESD is a rare lysosomal disorder affecting intrahepatic colesterol hydrolysis lacking a specific treatment. Ezetimibe, a cholesterol absorption inhibitor, should represent an option never delivered as monotherapy.

Methods: A 15 yrs old male patient affected by CESD, diagnosed on the basis of liver steato-fibrosis and LIPA gene mutation, was treated with 10 mg/die for 2 yrs. Biochemical parameters were checked at basal time,6 months,1, 2 yrs since starting therapy. Liver enzymes, lipid profile were tested by standard methods. Inflammatory markers of lipid peroxidation interleukins 1beta (IL1β) and 6 (IL6) were evaluated using western blot analyses. Tranforming growth factor-beta1 (Tgf-beta1), choosen as hepatic fibrosis parameter was evaluated by elisa kit. The serum protein adducts Malonaldehyde (MDA) and 4-hydroxynonenal (HNE) were analyzed to test the oxidative damage. Liver elastograpy employed to test liver fibrosis.

Results: Liver enzymes normalized by 6 months of therapy; total cholesterol and LDL-C decreased by 30% and 25 % respectively at the same time. IL1β and IL6 decreased after one year of treatment. As well the oxidative markers malonaldehyde (MDA) and 4-hydroxynonenal (HNE) resulted 4,3 ± 0.3 and 6.9± 0.7 AFU/mg protein in basal condition and 3.8±0.5 and 5.1±0.5 AFU/mgprotein after 1 yr treatment, so showing a normal value. Tgf-beta1 did not change in agreement with unchanged elastografic fibrosis.

Conclusion: Emphasis for CESD will lead to the disorder being recognized and correctly treated. Present results underline the well tolerated successful outcome reached by ezetimibe monotherapy in reducing lipoprotein levels, normalizing transaminases, cytokines and oxidative stress parameters.

Poster Session

INFLUENCE OF PHYSICAL TRAININGS ON BLOOD OXYGEN TRANSPORT INDICES AND ENDOTHELIAL FUNCTION IN PATIENTS WITH STABLE ANGINA

Liudmila Yankovskaya

Grodno State Medical University, Grodno, Belarus

The aim of our study was to assess the efficiency of the medicinal treatment and bicycle trainings (BT) in patients with stable angina class II as for the indices of blood oxygen transport (BOT) and endothelial function. Group A included 16 patients which were treated with medicines. Group B included 10 patients which were treated with the same medicines plus BT for 14 days. BOT indices (pO2, pCO2, pH, Hb, metHb) were measured using micro-gas analyzer. The hemoglobin-oxygen affinity was determined according to the p50 index. Endothelium dependent vasodilatation (EDV) was measured by plethysmography. Plasma nitrite/nitrate level (NOx) was determined using Griess method.

Results: Maximally tolerated load increased only in group B by 27,8% ($p < 0,05$). The number of metabolic units also increased and was 7,80±0,47, which indicated improved assimilation of oxygen by tissues. «Double product», indirectly characterizing assimilation of oxygen by myocardium, increased by 12,3% ($p < 0,05$) in group B and did not change in group A. Indices of BOT did not change, p50 increased in group A after treatment. In group B pO2 after treatment was 13,4% ($p < 0,05$) higher than in group A. pH increased to 7,334±0,009 units, p50 increased by 3,1% ($p < 0,05$). In group A EDV became 14,04±1,23% ($p < 0,001$). In group B EDV became 17,70±1,73% ($p < 0,001$) and was 26% higher, than in group A. NOx increased ($p < 0,05$) only in group B and was 21,81±1,09 μmol/l.

Conclusion: BT improves more effectively oxygen supply of tissues and endothelial function as compared to medicinal treatment alone in patients with stable angina.

Poster Session

LIPO-RETRO-D-PEPTIDES: NEXT GENERATION OF THE HIGHMOST SELECTIVE THROMBIN INHIBITORS

Alexey Poyarkov, S. Poyarkova

Protein Chemistry Department, Institute of Bioorganic Chemistry and Petrochemistry of the Ukrainian National Academy of Sciences, Kyiv, Ukraine

The research is devoted to the investigation of structure-activity relationship (SAR) of novel inhibitors with a main target of the blood coagulation system enzyme - thrombin.

Chemical modification of the main retro-D-peptide sequence - D-Arg-D-Phe- by different aromatic, heterocyclic and aliphatic moieties led to discovery of lipo-retro-D-peptides - highmost selective thrombin inhibitors. Modification of the retro-D-peptide sequence - D-Arg-D-Phe- by fatty acids residues provided compounds which inhibit thrombin in the range of Ki from 2 to 0,1 μM and with selectivity of 200-6000 against trypsin.

It has been proved that the well-known surfactant LAE (Nα-Lauroyl arginine ethyl ester) is a competitive reversible thrombin inhibitor. It has been shown that LAE inhibits thrombin activity almost 20 times stronger than trypsin (Ki=18,9 μM). At the same time LAE is hydrolyzed by thrombin at pH 8,5 (kcat = 3,6 c-1) and trypsin (kcat=56 c-1) at the same pH. The LAE ability to activate plasmin at the same range of thrombin inhibition concentration deserves special attention.

These findings establish an important role of fatty moiety in structure of inhibitors in preferential selective binding and inhibition of thrombin active side.

Features of ion formation under the influence of laser emission under the conditions of MALDI-TOF mass spectrometry in the glycosylated protein thrombin and the related proteases trypsin and chymotrypsin were studied for the first time. In addition, the attempt to investigate features of thrombin-inhibitor interactions with MALDI TOF MS was made.

Poster Session

DETERMINATION OF THE PROOXIDANT-ANTIOXIDANT BALANCE IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY AND IN RENAL TRANSPLANT PATIENTS

Vera Markovic, T. Gojkovic, M. Kostic

University of Belgrade, Belgrade, Serbia

Introduction: Several studies results indicate that oxidative stress might contribute to renal disease development.

The aim of study: Determination of prooxidant-antioxidant balance(PAB) in patients with chronic renal insufficiency(HRI) and in renal transplant patients in purpose of identifying oxidative stress as risk factor in renal disease development.

Methods and material: Thirty-eight patients (12 patients with HRI and 26 renal transplant patients) and fifty healthy volunteers were chosen for this study. PAB, as indicator of oxidative stress state, was determined in plasma using spectrophotometric method. The absorbance of coloured compound, that is a product of reaction between 3,3',5,5'-tetramethylbenzidine and hydrogen-peroxide, was read at 450nm. The results are expressed in HKU.

Results: The results of this study indicate significant decreasing in anti-oxidative parameter values (enzyme SOD and SH-groups) of renal patients in comparison to control group, while the control group stated higher activity of antioxidative enzyme PON1. Increased values of SH-groups and decreased level of $O_2^{\cdot -}$ in patients with HBI indicate better antioxidative state in comparison to renal transplant patients.

Significant increase of the PAB value was observed in renal patients in comparison to control group, but there was no significant difference betwen the two groups of patiens. In control group, positive corelation was found between PAB and leucocytes and negative corelation with enzym paraoxonase. Positive corelation between PAB and DZO in patients group probably represents a compensatory organism response.

Conclusion: Measuring PAB might be useful for evaluation of oxidative stress state in patients with renal function failure.

Poster Session

PROINFLAMMATORY CIRCULATING FACTORS IN NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED TO METABOLIC SYNDROME

Diego Lucero[1], V. Zago[1], G. López[1], V. Miksztowicz[1], H. Fainboim[2], M. Graffigna[3], L. Gómez Rosso[1], G. Berg[1], R. Wikinski[1], F. Brites[1], L. Schreier[1]

[1]Lipids and Lipoproteins Laboratory, Clinical Biochemistry Depart.-INFIBIOC Research Institute, University of Buenos Aires, [2]Hepatology Service, Muñiz Hospital, [3]Endocrinology Division, Durand Hospital, Buenos Aires, Argentina

Non-alcoholic fatty liver (NAFL) is another component of the metabolic syndrome (MS) that it might be associated with alterations in proinflammatory factors which would contribute to cardiovascular disease risk. Our aim was to measure circulating inflammatory markers in NAFL associated to MS. We studied 68 patients with MS (ATP III). Abdominal ultrasonographies were performed by a single operator, and patients were divided into two groups according to the presence (**A**) or the absence (**B**) of NAFL. Blood was drawn after 12 hour fasting. Lipid profile and the levels of free fatty acids (FFA), adiponectin, TNF-α, CRPhs, VCAM-1 and ICAM-1 were measured in serum. Triglycerides and FFA were higher in **A** ($p< 0.05$). There were no differences in TNF-α, VCAM-1 and ICAM-1 between groups. Adiponectin presented a significant reduction in **A** (media±SD: 6.7±2.4 *vs* 9.0±6.2 µg/ml; $p=0.04$) independently of HOMA-IR, while CRPhs was higher (median, range: 2.2, 0.3-9.3 *vs* 1.2, 0.1-3.7 mg/l, $p< 0.02$) and correlated with all the inflammatory markers: TNF-α ($r=0.34$, $p< 0.02$), VCAM-1 ($r=0.29$; $p< 0.03$), ICAM-1 ($r=0.56$; $p< 0.01$), adiponectin ($r=-0.34$; $p=0.04$) and FFA ($r=0.35$; $p< 0.05$). Also, opposite correlations were observed between FFA and TNF-α ($r=0.36$; $p< 0.04$) and between FFA and adiponectin ($r=-0.40$; $p< 0.03$) even after adjusting by insulin-resistance factors. The increase in FFA influx from adipose tissue to the liver is closely linked to an inflammatory state. NAFL would constitute a low chronic inflammatory condition, represented in plasma by higher CRPhs levels and lower adiponectin concentration, independent of the presence of insulin resistance.

Poster Session

PARAMETERS OF OXIDATIVE STRESS AND ANTIOXIDATIVE PROTECTION IN PLASMA OF FANCONI ANEMIA PATIENTS

Jelena Joksic

Medical Biochemistry, Faculty of Pharmacy, Belgrade, Serbia

Introduction: Fanconi anemia is an autosomal-recessive disease characterized by genome instability, increased sensitivity to ROS, significant telomere shortening, progressive pancytopenia.

The role of plasma SOD out competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. An important role of SOD-3 recently established is slowing telomere shortening in every cell division.

Aim: Establishing activity of plasma SOD, level of prooxidative processes, and the relationship between these parameters and pathogenesis of the disease.

Material and methods: The research focused on 84 healthy individuals (61 children and 23 adults) 10 FA patients (children with disease) and 11 parents. Heparinized plasma as sample was used. SOD activity was determined by original method by Mirsa and Fridovich, which is based on SOD's ability to inhibit adrenalin autooxidation in alkaline environment. The level of prooxidative-antioxidative balance (PAB) was determined by method which uses 3,3',5,5' thetrametilbensidine reagent, and measures the concentration of H_2O_2 in antioxidative environment.

Results: Statistical analysis of obtained results pointed out significant decrease of SOD-3 activity, in FA patients (a_{pac}=26±22kU/L) and their parents (a_r=27±23 kU/L) compared to controls (a_{kg}=108±36 kU/L; a_{kgd}=133 ±30 kU/L) $p< 0.001$. There was no significant difference between FA patients and their parents. The levels of PAB in FA patients and their parents were significantly increased, $p=0.05$ (C_{kg}=343±200 HKU *vs.* C_r=467±84 HKU; C_{kgd}=372±197 HKU *vs.* C_{pac}=593±247 HKU).

Conclusion: Decreased plasma SOD activity in FA patients and their parents indicate strong influence of mitochondrial inheritance, and could be related to accelerated telomere shortening, and therefore decreased bone marrow cell-life.

Poster Session

ACUTE DECREASE OF ANTI-HEAT SHOCK PROTEIN 27 AND ACUTE INCREASE OF HIGH SENSITIVE C-REACTIVE PROTEIN AFTER PERCUTANEOUS CORONARY INTERVENTION

Majid Ghayour-Mobarhan[1], M. Mouhebati[2], H. Falsoleiman[2], S. Tavallei[1], R. Paydar[2], H. Pourghadamyari[1], G. Ferns[3]

[1]Cardiovascular Research Center and Nutrition and Biochemistry, [2]Cardiovascular Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, [3]University of Keele, Staffordshire, UK

Objective: According to new findings about anti-heat shock protein (HSP) 27 as a novel risk factor for atherosclerosis, we aimed to evaluate anti- HSP 27 antibody titers and high-sensitive C-reactive protein (hs-CRP) in patients undergoing percutaneous intervention (PCI) before and after the procedure.

Method: Blood samples were collected from all subjects 24 hours before and 24 h after PCI. Anti-Hsp27 IgG titers were determined using an in-house enzyme-linked immunoasorbent assay (ELISA) and hs-CRP was measured by a PEG-enhanced immunoturbidimetry method with an Alycon analyzer.

Results: Median anti-HSP27 antibody levels for patients (n=145) before PCI were 0.29 (0.22-0.46) and after PCI 0.24 (0.17-0.38), which were significantly decreased after PCI ($P < 0.001$). Moreover, hs-CRP levels were increased significantly from before PCI 2.37 (1.23-7.32) to 61.54 (10.33-82.11) after PCI, respectively ($P < 0.001$). The comparison of anti-HSP27 antibody and hs-CRP levels between those patients that drug-eluting stents were implanted for them and those that bare-metal stent were used showed no significant difference after PCI ($P>0.05$). In these patients, antibody titers to HSP27 levels were not correlated with clinical and biochemical parameters ($P < 0.05$).

Conclusion: Severe increase of hs-CRP and decrease of anti-HSP27 after PCI proved the acute augmentation of inflammatory process after PCI which is a proatherosclerotic and restenotic factor. Suggesting, that anti-inflammatory medications including statins and aspirin might be a useful defensive approach.

Keywords: Heat shock protein, antibody, Percutaneous intervention.

Poster Session

HAEMODYNAMIC EFFECTS OF LACTOTRIPEPTIDES FROM CASEIN HYDROLYSATE IN MEDITERRANEAN HEALTHY SUBJECTS: A RANDOMIZED, DOUBLE-BLIND, CROSS-OVER TRIAL

A. Cicero, **Martina Rosticci**, M. Veronesi, S. Bacchelli, E. Strocchi, E. Grandi, C. Borghi

Internal Medicine, Aging and Kidney Disease Dept., University of Bologna, Bologna, Italy

Background: Contrasting data partially support the antihypertensive efficacy of Ile-Pro-Pro / Val-Pro-Pro lactotripeptides derived from casein hydrolysate.

Aim: We carried out a randomized, double-blind, cross-over clinical study to investigate the antihypertensive efficacy of a short-term treatment with lactotripeptides in 55 subjects with normal or high-normal blood pressure (BP), in term of reduction of 24 hour ambulatory blood pressure measurement (ABPM), and plasma renin activity.

Methods: After 4 weeks of dietary stabilization, patients were allocated to treatment with a fruit juice added with 3 mg lactotripeptides or placebo. After a 4 week wash-out period, they were then assigned to the alternative treatment for further 4 weeks.

Results: No significant change in body weight, BMI and waist circumference was observed in both treatment group. Overall no significant difference has been observed in office systolic, diastolic and pulse pressure when comparing baseline data with those post-treatment. Repeating the analysis by basal BP level, a mild but significant reduction in SBP (-1.7±2.3 mmHg; p=0.002) has been observed only in subjects with high-normal BP after treatment with lactotripeptides. As it regards 24h BP measurement, after lactotripeptides treatment only, the subjects experienced a significant reduction in diurnal DBP (-1.6±5.4 mmHg, p=0.042), in diurnal MBP (-2.1±5.9 mmHg, p=0.19), in 24-hour (-5.4±14.2 mmHg, p=0.011) and diurnal (-7.1±19.2 mmHg, p=0.014) DBP value measurements over the normal. No modification has been observed as it regards plasma renin activity and aldosteronemia.

Conclusion: Diurnal DBP is significantly reduced by a lactrotripeptides supplementation in untreated Mediterranean subjects with normal or high-normal blood pressure.

Poster Session

EFFECT OF HYPERCHOLESTEROLEMIA AND TREATMENT WITH STATINS ON THE ANTIHYPERTENSIVE EFFICACY TO OLMESARTAN AND AMLODIPINE: A RETROSPECTIVE CLINICAL TRIAL

Fabrizio Imola, A. Cicero, M. Veronesi, E. Strocchi, E. Tartagni, C. Borghi

Internal Medicine, Aging and Kidney Disease Dept., University of Bologna, Bologna, Italy

Background: Preclinical and epidemiological suggest that hypercholesterolemia could influence the antihypertensive efficacy of drug acting on the renin-angiotensin-aldosteron system (RAAS) or on other targets.

Aim: To retrospectively evaluate if the normalization of cholesterolemia by statin treatment is associated to a different antihypertensive answer to angiotensin 1 (AT1) receptor antagonists or calcium antagonists.

Methods: We retrospectively evaluated the pressure control of 42 patients preliminarily treated with AHA step 2 diet and Olmesartan 20 mg (N. 21) or Amlodipine 5 mg (N. 21), and after 6 weeks with Rosuvastatin 20 mg. We selected those patients either affected by first degree hypertension and polygenic hypercholesterolemia, untreated for both cardiovascular disease risk factors, and in primary prevention for cardiovascular disease.

Results: At the baseline the patients were comparable as it regards their blood pressure level and their LDL-cholesterolemia. After the first period of treatment, Olmesartan 20 mg treated patients experienced a more relevant blood pressure decrease than Amlodipine 5 mg treated ones (SBP: -15±4 mmHg vs. -10±5 mmHg, p=0.013; DBP: -12±5 mmHg vs. -7±4 mmHg, p=0.048). However, after cholesterolemia optimization by statin treatment the previously observed difference in efficacy of the two antihypertensive drugs disappeared.

Conclusion: Our preliminary and retrospective study support the hypothesis that hypercholesterolemia induce up-regulation of AT1- receptor, so increasing the antihypertensive efficacy of AT1 receptor blockers. A prospective double-blind, randomized clinical trial is starting in our centre to confirm or less this preliminary observation.

Poster Session

MOBILIZATION OF CD133+ PROGENITOR CELLS AND ITS CORRELATION TO INTERLEUKIN-8 IN ACUTE CEREBRAL INFARCTION

Daniela Franz[1], D. Sepp[1], I. Ott[2], P. Zepper[1], S. Sadikovic[1], R. Feurer[1], L. Esposito[1], B. Hemmer[1], H. Poppert[1]

[1]Neurology, [2]Cardiology, Technische Universitaet Muenchen, Munich, Germany

Background: Progenitor cells (PCs) support the regeneration process in tissue affected by ischemia by stimulating neovascularisation and reendothelialisation. Interleukin(Il-)8 acts as a chemoattractant in inflammatory processes and represents a potent pro-angiogenic factor. Results of an earlier study of our research group suggested that serum levels of Il-8 are associated with the number of circulating PCs in acute myocardial infarction. In this study we aimed to evaluate an interaction between inflammatory response and the mobilization of PCs in acute cerebral infarction.

Methods: 39 patients with radiologically proven acute cerebral infarction were included. The number of circulating CD133+PCs in peripheral venous blood was analyzed by flow cytometry. Levels of Il-8 were measured with cytometric bead array-kits. Blood samples were taken within 24 hours after symptom onset as well as on day 5 and 7.

Results: Numbers of CD133+PCs were elevated on day 5 and day 7 (median[range] 0,32[0-2]cells/µl; 0,29[0-2]cells/µl) compared to the initial value (median[range] 0,12[0-2]cells/µl). On day 7 serum levels of Il-8 (median[range] 12,3[0-60,4]pg/ml) correlated significantly with the number of CD133+PCs (r=0,342, p=0,03). Serum levels of Il-8 at admittance and on day 5 (median[range] 14,9[0-126,3]pg/ml; 12,1[0-91,1]pg/ml) showed no significant correlation to CD133+PCs.

Discussion: This study showed a mobilization of CD133+PCs after cerebral infarction and an association between the number of circulating CD133+PCs and Il-8. Hence it can be speculated that Il-8 contributes to the progenitor cell mobilization after acute cerebral infarction. This could represent a potential beneficial effect of the inflammatory reaction after stroke.

Poster Session

ARTERIAL STIFFNESS IN PAROXYSMAL ATRIAL FIBRILLATION: RELATIONSHIP WITH MICRO- AND MACROVASCULAR ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE

Suresh Krishnamoorty, C. Khoo, H.S. Lim, G.Y. Lip

University Department of Medicine Centre for Cardiovascular Sciences, City Hospital, Birmingham, UK

Background: Increased pulse pressure (PP, surrogate of arterial stiffness) is associated with atrial fibrillation. It is not clear if arterial stiffness is

(i) mediated by micro- and macro-vascular endothelial dysfunction (ED) and

(ii) related to co-existent cardiovascular risk factors.

Methods: We studied 42 patients with paroxysmal atrial fibrillation (PAF), 45 hypertensives and 45 healthy controls. Macrovascular ED was measured by the change in aortic augmentation index (AIx), using carotid artery applanation tonometry in response to inhaled salbutamol and sublingual glyceryl trinitrate. Microvascular ED was measured by cutaneous laser Doppler flowmetry (LDF) in response to acetylcholine (Ach, endothelium-dependent) and sodium nitroprusside (endothelium-independent). Arterial stiffness was measured using carotid-femoral pulse wave velocity (PWV). 'Reservoir pressure' (Pr, derived from MATLAB algorithm) describes aortic 'cushioning' properties.

Results: At baseline, PP, PWV, Pr and AIx were significantly higher in patients with PAF and hypertension compared to healthy subjects, but not between the patient groups.

	Healthy (n=45)	Disease controls (n=45)	PAF (n=42)	p
Age (in years)	56 (10)	57 (8)	59 (13)	0.34
Males (n)	25	35	29	0.12
SBP (mm of Hg)	126 (9)	132 (16)*	136 (15)*	0.004
PP (mm of Hg)	48 (8)	53 (15)*	59 (12)*	0.001
AIx Baseline	28 (22-37)	34 (25-42)*	39 (31-42)*	0.01
%ΔAIx Salbutamol	30 (15-44)	16 (6-30)*	10 (6-27)*	0.001
PWV (m/s)	5.8 (0.9)	6.4 (1.1)*	6.4 (1.5)*	0.03
% ΔLDF Ach	125 (99-138)	74 (41-108)*	95 (61-115)*	0.01
% ΔLDF SNP	95 (65-120)	67 (43-94)*	83 (55-122)*	0.01
Reservoir pressure	122 (14)	129 (15)*	129 (12)*	0.03

[Table 1]

Similarly, the change in AIx with salbutamol (%ΔAIx) and ACh-induced changes in LDF were significantly lower in patients with PAF and hypertension compared to healthy subjects, but no differences seen between disease groups. PWV correlated with changes in AIx (r=-0.186, p=0.03), LDF (r=-0.249, p=0.01) and age (r=0.404, p< 0.01). Pr correlated with PWV (r=0.367, p< 0.01) and age (r=0.209, p=0.03) but not to changes in AIx and LDF.

Conclusion: Arterial stiffness (PWV) in patients with PAF is mediated by macro- and microvascular ED, however these changes in ED are related to co-existent risk factors rather than the arrhythmia per se.

Poster Session

VITAMIN K2 SUPPLEMENTATION REDUCES THE ELEVATED INACTIVE FORM OF THE CALCIFICATION INHIBITOR MATRIX GLA PROTEIN IN HEMODIALYSIS PATIENTS

Thilo Krueger[1], R. Westenfeld[2], G. Schlieper[1], E. Cranenburg[3], S. Heidenreich[4], S. Holzmann[5], C. Vermeer[3], M. Ketteler[6], J. Floege[1], L. Schurgers[3]

[1]Division of Nephrology and Clinical Immunology, University Clinic of the RWTH Aachen, Aachen, [2]Department of Cardiology, Heinrich-Heine-University Duesseldorf, Düsseldorf, Germany, [3]Cardiovascular Reseach Institute and VitaK BV, University Maastricht, Maastricht, The Netherlands, [4]Kfh Dialyse, Aachen, [5]Dialysezentrum, Erkelenz, [6]Department of Nephrology, Klinikum Coburg, Coburg, Germany

Vascular calcification is a potent risk factor for cardiovascular death in hemodialysis (HD) patients. Matrix Gla protein (MGP) is a vitamin K-dependent protein and a strong inhibitor of vascular calcification. Vitamin K-deficiency leads to uncarboxylated, inactive MGP (ucMGP). The aim was to determine ucMGP levels in HD patients and to investigate whether dietary vitamin K2 supplementation reduces ucMGP levels in this high-risk population.

Controls (n=102), HD patients (n=53). After baseline blood drawing, HD patients randomly to received 45, 135 or 360µg vitamin K2 once daily for six weeks. Circulating ucMGP and uncarboxylated osteocalcin (ucOCN) levels were measured as independent indicators of extrahepatic carboxylation activity.

HD patients displayed greatly enhanced ucMGP serum levels compared to controls (2013 vs. 432 ; $p< 0.01$). Vitamin K2 supplementation induced a dose-dependent decrease of mean ucMGP serum levels by 11% ($p< 0.5$), 16% ($p< 0.01$), and 28% ($p< 0.001$). The proportion of responders, as judged by reduced ucMGP levels following vitamin K2 supplementation, increased with higher vitamin K2 supplementation from 80% at 45µg up to 100% at 360µg. ucOCN serum levels mirrored the changes in ucMGP levels, being reduced by 9% (n.s.), 11% (n.s.) and 48% (*$p< 0.05$) in the three treatment groups. Vitamin K2 supplementation was well tolerated.

The inactive form of the calcification inhibitor MGP is greatly increased in HD patients. Six weeks of vitamin K2 supplementation suffice to reduce ucMGP levels in HD patients suggesting that disturbed calcification inhibitory activity in the vasculature may be improved by dietary vitamin K2 supplementation.

Poster Session

THE LEVEL OF ATHEROSCLEROTIC DAMAGE OF THE MAGISTRAL AND PERIPHERAL ARTERIES IN CHRONIC RENAL FAILURE PATIENTS

Ievgeniia Frolova, O. Kuryata

Dnipropetrovsk State Medical Academy, Dnipropetrovsk, Ukraine

Uraemic dyslipidemia is the risk factor of development of atherogenesis in patients (pts) with chronic renal failure (CRF), that can lead in subsequent to development of cardiovascular diseases (CVD) at this category of pts. We aimed to evaluate the level of atherosclerosis damage of the magistral arteries(MA) and peripheral arteries(PA) depending on the lipid profile (total cholesterol (TC) and triglycerides (TG)) at pts with CRF(glomerular filtration rate(GFR) ≤60>15 ml/min/1,73m2). 15 pts (female) with CRD(mean GFR 39,91±5,38 ml/min/1,73m2), mean age - 39,13±2,16 years without established CVD. The level of atherosclerosis damage of the MA and PA were estimated by determination of the carotid intima-media thickness(IMT) and the ankle-brachial index(ABI) by using Echo-Doppler ultrasound system. At all pts the lipid profile was determined in blood by ELISA. Among the inspected patients the atherosclerotic changes on carotids were registered in 8(53,33%): 4(50%) pts had increase IMT(1,0≤1,3 mm), 4(50%) pts had atherosclerotic platelets(IMT >1,3 mm). 6(75%) pts had bilateral damages of carotids. Increased IMT correlated to GFR(r=-0,38; p

Poster Session

SIMVASTATIN RETARDS PROGRESSION OF AORTIC VALVE STENOSIS IN THE ELDERLY PATIENTS WITH CHRONIC HEART FAILURE AND PRESERVED SYSTOLIC FUNCTION

Olga Mytrokhina, O. Kuryata

Dnipropetrovsk State Medical Academy, Dnipropetrovsk, Ukraine

Hypercholesterolemia and C-reactive protein (CRP) predicts severity and progression of aortic stenosis (AS) in the elderly patients (pts) with chronic heart failure (CHF). Statins have shown pleotropic effects, however the role of statins in pts with CHF and AS remains debatable. We aimed to evaluate the effects of simvastatin 10 mg/day on progression of AS in the elderly pts with CHF and preserved systolic function. 34 pts (21 M, 13 F, mean age - 74,97±2,75 years) with CHF NYHA I-III class and preserved systolic function and with asymptomatic AS were enrolled. Aortic valve area (AVA) was calculated by Doppler echocardiography in all pts. CRP and total cholesterol (TC) levels were determined in the blood. 20(58,8%) pts received the standard treatment and simvastatin 10 mg/day (1 group), 14(41,17%) (2 group) - received only the standard treatment for 24 weeks. Increased TC and CRP levels correlated to AS (r=0,35and r=0,44; p

Poster Session

RAPID AND ACCURATE GENETIC DIAGNOSIS BY LIPOCHIP® IN UK FH PATIENTS

Lourdes Palacios[1], M. Stef[1], A. Taylor[2], S.E. Humphries[3], N. Cuevas[1], C. McAnulty[4], D. Bourn[4], D. Tejedor[1], R.D. Neely[5]

[1]Progenika Biopharma SA, Derio, Spain, [2]Regional Molecular Genetics Laboratory, Great Ormond Street Hospital for Children, [3]Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, Royal Free and University College London Medical School, London, [4]Nothern Genetics Service, Newcastle upon Tyne NE BZ, [5]Lipid and Metabolic Clinic, Royal Victoria Infirmary, Newcastle upon Tyne NE LP, UK

Familial Hypercholesterolemia (FH) is an autosomal dominant disorder mostly caused by mutations in the LDLR gene but also in the APOB and PCSK9 genes.

The DNA-array system LIPOchip® detects point mutations in the LDLR, APOB and PCSK9 genes as well as Copy Number Change (CNC) in the LDLR gene. LIPOchip® v8.0 includes the 251 mutations (242 LDLR, 3 APOB and 6 PCSK9 mutations) most prevalent in Spain, Netherlands, Norway, Italy and the United Kingdom.

We evaluated the performance of LIPOchip® in a blinded study of 126 DNA samples from patients with Definite or Possible FH according to the Simon Broome criteria recruited for the Department of Health FH DNA Pilot Study. All samples had previously been tested using a commercial ARMS kit followed by screening in all exons by SSCP/dHPLC/direct-sequencing and MLPA if no was mutation detected. 62 patients were known to have mutations. The analysis by the LIPOchip® (Progenika Biopharma) identified mutations in 37 patients. On sequencing of the LIPOchip® negative samples, mutations were found in a further 28 cases. In total, 42 different mutations were detected in 65 patients, the most frequent being: R3500Q in APOB, D461N in LDLR and D374Y in PCSK9, all of them detected by the chip. For the mutation positive samples, the detection rate by the chip was 56.9%, with a sensitivity of 100% for the mutations on the chip. This approach allows rapid screening of large numbers of samples improving the efficiency of testing for the most common European mutations associated with FH.

Poster Session

THE EFFECT OF ALISKIREN ON ATHEROSCLEROSIS DEVELOPMENT IN APOE*3LEIDEN.CETP TRANSGENIC MICE WITH AND WITHOUT TREATMENT WITH ATORVASTATIN

Susan Jordaan[1,2], J.W.A. van der Hoorn[1], L.M. Havekes[1,2], G. Liau[3], J.W. Jukema[2], H.M.G. Princen[1]

[1]TNO BioSciences, [2]Dep. of Cardiology, Leiden University Medical Center, Leiden, The Netherlands, [3]Novartis Institutes for BioMedical Research, Cambridge, MA, USA

Objectives: Aliskiren is the first commercially available, orally active, non-peptide-like direct renin inhibitor approved to treat hypertension. The renin angiotensin system may be a significant contributor to the development of hypercholesterolemia-induced atherosclerosis. The aim of the study is to evaluate the anti-atherosclerotic effects of aliskiren alone and in combination with atorvastatin.

Methods: APOE*3Leiden.CETP mice (n=14-17 per group) were fed a western-type diet (containing 0.25% cholesterol) alone or were treated with either aliskiren (15 mg/kg/d), atorvastatin (3.5 mg/kg/d) or a combination of aliskiren and atorvastatin. Effects on systolic blood pressure (SBP), plasma lipid levels, inflammation markers, and atherosclerosis size and composition were assessed.

Results: Compared to the control, aliskiren reduced SBP (-17 to -24%; $p< 0.05$) and atorvastatin reduced cholesterol levels (on average -21%; $p< 0.01$). A decrease in severe lesions (as % of all lesions) was revealed with atorvastatin (46% vs 66% in control, $p< 0.01$). Aliskiren (-40%, $p< 0.05$), atorvastatin (-61%, $p< 0.001$) and the combination treatment (-69%, $p< 0.001$) were found to reduce the total lesion area. Only the combination group showed a decrease in the amount of atherosclerotic lesions per cross section (-17%; $p< 0.05$). Results of inflammation markers and lesion composition are pending.

Conclusions: The reduction in SBP with aliskiren, as well as the reduction in cholesterol with atorvastatin reflected the expected effects of treatment. Aliskiren was found to reduce the development of atherosclerosis, but did not potentiate the anti-atherosclerotic effect of atorvastatin. Only the combination treatment of aliskiren and atorvastatin decreased total atherosclerotic lesion numbers.

Poster Session

RECONSTITUTED HIGH DENSITY LIPOPROTEIN INHIBITS RESTENOSIS IN BALLOON-INJURED RAT CAROTID ARTERY

Jo Woon Yi Lee[1], J.Y. Lee[1], J.Y. Kim[1], H.J. Ku[1], B.-S. Lee[1], J.E. Park[1], K.-Y. Kim[2]

[1]Division of Cardiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, [2]2 Protein Research Lab, CRC, Green Cross Corp., Seoul, Republic of Korea

Background and aim: Maintaining low level of low density lipoprotein (LDL) and high level of high density lipoprotein (HDL) in plasma is the most important factor in the prevention of cardiovascular diseases. We tried to investigate the inhibitory effects of reconstituted HDL (rHDL) on restenosis and its mechanism of action.

Methods and results: rHDL was prepared with plasma-derived apoA-I and soybean PC by a molar ratio of 1 to 150. To induce neointima formation, the endothelium was denuded with balloon catheter in the right carotid artery of SD rats (n=10). rHDL administration was started 4 hr before surgery and continued two consecutive days after carotid injury. Two weeks later, arteries were isolated from rats and neointima formation was assessed by H&E staining. Intima to media ratios was 20-fold in 40 mg/kg rHDL treated group ($p=0.4$) and 25-fold lower in 80 mg/kg rHDL treated group ($p< 0.006$) than in control group. Immunohistochemistry revealed that significant reduction of Ki-67 signal in neointima but increase of the signal in endothelial area. HO1 is increased in neointima of all injured groups. Overall expression of HSP27 was not changed but phosphorylation of HSP27 was not shown in those of rHDL treated groups. In addition, strong CD68 signals in carotid arteries are dramatically diminished in injured lesions of rHDL treated groups.

Conclusions: rHDL effectively stabilized restenosis by inhibiting celll cycle and inflammation in injured lesions. HO1 and phosphorylation of HSP27 might play a role in ameliorating the progression of restenosis in balloon-injured rat carotid artery.

Poster Session

FUNCTIONAL AND STRUCTURAL PROFILING OF THE HUMAN THROMBOPOIETIN GENE PROMOTER

C. Dördelmann[1], **Ralph Telgmann**[2], S. Hasenkamp[3], B. Schröer[2], C. Hagedorn[4], P. Baumgart[5], P. Kleine-Katthöfer[6], M. Paul[7], E. Brand[8], S.-M. Brand-Herrmann[2]

[1]Institute for Analytical Sciences, Dortmund, [2]Molecular Genetics of Cardiovascular Disease, Leibniz-Institute for Arteriosclerosis Research, Münster, Germany, [3]University of Gothenburg, Gothenburg, Sweden, [4]University Witten/Herdecke, Witten, [5]Clemenshospital GmbH Münster, [6]Franziskus-Hospital Münster, Münster, Germany, [7]Maastricht University, Maastricht, The Netherlands, [9]University Clinic Münster, Münster, Germany

Human thrombopoietin (TPO) is involved in cardiovascular disease (CVD) as it regulates megakaryocyte development and enhances platelet adhesion/aggregation. *THPO* promoter structure still being unresolved, we found by PCR that *THPO* transcription is cell line-dependently initiated at two alternative promoters, which we designated P1a and P1. We subsequently resequenced these portions in 95 and 46 patients with CVD, respectively, and identified eight genetic variants (-1450/del58bp, C-920T [rs2855306], A-622G, C-413T [rs885838], C+5A, C+102A, G+115A, and C+135T). After subcloning of 1032 bp fragments of *THPO* P1 in a luciferase reporter vector, five molecular haplotypes (MolHaps1-5) were, respectively, observed: [A^{-622}-C^{-413}-C^{+5}-G^{+115}; wildtype (wt)], [A^{-622}-T^{-413}-C^{+5}-G^{+115}], [G^{-622}-T^{-413}-C^{+5}-G^{+115}], [A^{-622}-C^{-413}-A^{+5}-G^{+115}], [A^{-622}-C^{-413}-C^{+5}-A^{+115}], and analysed in HEK293T and HepG2 cells. While MolHaps 2, 4, 5 were significantly more active than wt (all P-values ≤0.01), MolHap 3 exerted a substantial loss of promoter activity (P< 0.0001 in HEK293T; P=0.001 in HepG2, compared to wt). EMSAs revealed that A-622G and C-413T in single assays differed from MolHaps in their DNA:protein interaction patterns and Supershift assays identified C/EBPδ as binding protein exclusively for the -622A allelic portion. We redefined the transcriptional organisation of *THPO* and conclude that the P1 promoter is differentially regulated by complex genetic constellations.

Poster Session

RELATIONSHIP BETWEEN LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND INFLAMMATION IN CORONARY HEART DISEASES

Guoping Tian, G. Xig

Department of Cardiology, The Second Affiliated Hospital of Nanhua University, HengYang, China

Objective: To investigate relationship between serum lipoprotein-associated phospholipase A_2 (Lp-PLA_2) levels and severity of inflammation in coronary heart diseases (CHDs).

Methods: All persons(n＝140) were divided into control group(n＝38), stable angina pectoris(SAP) group (n＝41), unstable angina pectoris(UAP) group(n＝30) and acute myocardial infarction(AMI) group(n=31). Blood samples for measurement of Lp-PLA_2, CRP and IL-6 were taken before coronary angiography.

Results: Serum Lp-PLA_2 levels in CHDs were significantly higher than that in controls (P< 0.01). Compared with SAP group, the levels were also significantly increased in UAP group and AMI group (P< 0.01), higher levels remained significant after adjustment for traditional risk factors; No statistical significance was found in Lp-PLA_2 levels between patients with AMI and UAP (P>0.05); Serum Lp-PLA_2 level was positively correlated with CRP (r＝0.722, P< 0.01),and IL-6 level (r＝0.665, P< 0.01) by bivariate correlation analysis.

Conclusion: The serum levels of Lp-PLA_2 may be used as a parameter to predict severity of inflammation in coronary heart diseases.

Keywords: Lipoprotein-associated Phospholipase A_2; Coronary Heart Disease; Inflammation

Poster Session

ROLES OF THE METABOLIC SYNDROME AND CORONARY ATHEROSCLEROSIS IN SUBCLINICAL INFLAMMATION

Philipp Rein[1,2,3], C.H. Saely[1,2,3], S. Beer[1,2,3], A. Vonbank[1,2,3], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: The metabolic syndrome (MetS) and stable coronary artery disease (CAD) frequently coincide; the individual contributions of these entities to subclinical inflammation are unknown.

Methods: We enrolled 1010 consecutive patients undergoing coronary angiography for the evaluation of suspected or established stable CAD. The MetS was defined according to the AHA revision of the NCEP ATP-III criteria; coronary stenoses with lumen narrowing ≥50% were considered significant.

Results: From our patients 564 (55.8%) had significant CAD and 459 (45.4%) had the MetS; the prevalence of significant CAD was higher in patients with the MetS than in subjects without the MetS (59.5% vs. 52.8%; p=0.034). Serum CRP did not differ significantly between patients with significant CAD and subjects without significant CAD (p=0.706), but it was significantly higher in MetS patients than in those without the MetS (p< 0.001). Regarding the individual MetS traits, the MetS criteria low HDL-C (p< 0.001), large waist circumference (p=0.007) and high glucose (p=0.023) but not the high triglycerides (p=0.813) and high blood pressure criteria (p=0.170) proved significantly associated with CRP. When all MetS traits were entered simultaneously into one ANCOVA model, only the HDL-C criterion independently of age, gender, LDL-C, smoking, cardiovascular medications and of all other MetS criteria proved independently associated with CRP (F=44.19; p< 0.001).

Conclusions: CRP is strongly associated with the MetS but not with angiographically diagnosed coronary atherosclerosis. The overall association of the MetS with subclinical inflammation is driven by the low HDL cholesterol feature.

Poster Session

A NOVEL ANTHROPOMETRIC MEASURE FOR PREDICTING ALL-CAUSE MORTALITY

Andreas Rosenblad, J. Leppert, G. Nilsson

Center for Clinical Research Västerås, Uppsala University, Västerås, Sweden

Purpose: To compare the predictive ability for all-cause mortality of the novel anthropometric measure Waist-Hip-Height Ratio (WHHR) with Body Mass Index (BMI), Waist Circumference (WC), Waist-Hip Ratio (WHR) and Waist-Height Ratio (WHtR).

Methods: Men and women aged 40 and 50 from the county of Västmanland, Sweden were examined during the years 1990-99 (participation rate=48%). All-cause mortality was followed up until August 2008. For persons with complete anthropometric measures (n=33493, men=48%), Cox regression was performed for BMI, WC, WHR, WHtR and WHHR (defined as WHR divided by height). Multivariate adjustment added age, diabetic status, smoking, systolic and diastolic blood pressure, heart rate and glucose level. The predictive ability was calculated with a Pseudo R^2-measure and compared to WHHR using bootstrap.

Results: During follow up 1301 persons (4%, men=58%) died. The predictive ability was highest for WHHR followed by WHR, WHtR, WC and BMI. The difference was usually significant or close to significant using a one-sided p-value< 0.05.

Conclusions: WHHR performs better than WHR, WHtR, WC and BMI in predicting all-cause mortality.

	Univariate				**Multivariate (change from model without anthropometric measure)**			
	Men		***Women***		***Men***		***Women***	
Anthropometric Measure	Pseudo R^2	P-value	Pseudo R^2	P-value	Pseudo R^2 increase	P-value	Pseudo R^2 increase	P-value
WHHR	0.142	ref.	0.163	ref.	0.020	ref.	0.023	ref.
WHR	0.117	0.023	0.146	0.098	0.016	0.100	0.019	0.175
WHtR	0.075	<0.001	0.125	0.041	0.007	0.014	0.014	0.089
WC	0.047	<0.001	0.105	0.010	0.004	0.010	0.010	0.051
BMI	0.014	<0.001	0.053	<0.001	0.000	0.007	0.002	0.012

[Predictive ability of anthropometric measures]

Poster Session

PREGNANCY OUTCOMES IN FAMILIAL HYPERCHOLESTEROLEMIA WITH SPECIAL EMPHASIS ON PRETERM DELIVERY, BIRTH WEIGHT AND CONGENITAL MALFORMATIONS. A REGISTRY-BASED STUDY

Ieva Toleikyte[1], K. Retterstøl[2], T.P. Leren[3], P.O. Iversen[1]

[1]Department of Nutrition, Faculty of Medicine, University of Oslo, [2]Lipid Clinic, [3]Medical Genetics Laboratory, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Background and aims: Circumstantial findings have linked hypercholesterolemia to preterm delivery and offspring with low birth weight, but firm data are lacking. Hence, the aim of this study was to examine whether women with familiar hypercholesterolemia (FH) have a higher risk of adverse birth outcomes compared to women in the general population.

Study design and subjects: We identified 1,871 genetically verified FH women of fertile age from the Medical Genetics Laboratory-Registry at Oslo University Hospital. This data-set was coupled to that of the Medical Birth Registry of Norway, identifying women who had been pregnant and their birth outcomes between 1967 and 2006. The obtained data were compared to corresponding data from the general population in Norway, comprising about 2.3 million births, for the same period.

Results: The registry-match resulted in 2,319 births of 1,102 women with heterozygous FH. Compared with the general population the FH population did not have significantly higher risk of

(i) giving birth prematurely (< 37 gestational weeks),

(ii) delivering children with low birth weight (< 2,500 g), or

(iii) having children with congenital malformations.

The introduction of statins in 1992 in the treatment of hypercholesterolemia did not significantly increase the incidence of the three outcomes (i-iii) in the FH population, when comparing the period 1979-1991, i.e. before and the period 1999-2006, i.e. after statin introduction.

Conclusion: FH women are apparently not at a higher risk of either preterm or low birth weight outcomes or births with congenital malformations than are women in the general population.

Poster Session

PROTHROMBOGENESIS IN SUBJECTS WITH METABOLIC SYNDROME AND CENTRAL OBESITY

Azlina A Razak, T.S. Tengku Ismail, M.R. Mohamad Taib, M. Mustafa Kamal, S. Abd Muid, M.T. Osman, K. Yusoff, H. Nawawi

Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Malaysia

Background: Metabolic syndrome (MS) represents a cluster of abnormalities associated with an increased risk of coronary heart disease which may be partially explained by enhanced prothrombogenesis.

Objective: The aim of the study was to evaluate the levels of prothrombogenic markers in MS subjects with different glycaemic status and centrally obese subjects.

Methods: A total of 262 subjects (Mean±SD : 52±11, 90 Males) were recruited and divided into 5 groups: MS with normoglycaemia (MSNG), MS with impaired fasting glucose (MSIFG), MS with diabetes (MSDM), central obesity without MS (OBXMS) and non-MS control (NC). In addition, MSNG, MSIFG and MSDM were grouped as all MS group with a total number of 157. The blood levels of fibrinogen, homocysteine, tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were evaluated.

Results: MSNG group compared to NC group had higher fibrinogen ($p < 0.05$), homocysteine ($p < 0.005$), tPA ($p < 0.05$) and PAI-1 ($p < 0.05$). MSIFG group compared to NC group had higher homocysteine ($p < 0.05$) and tPA ($p < 0.005$). MSDM group compared to NC group had higher homocysteine ($p < 0.05$), tPA ($p < 0.001$) and PAI-1 ($p < 0.05$). In addition, All MS group compared to NC group had higher homocysteine ($p < 0.005$), tPA ($p < 0.001$) and PAI-1 ($p < 0.01$). OBXMS group compared to NC group had higher homocysteine ($p < 0.05$).

Conclusions: MS irrespective of glycaemic status have enhanced prothrombogenesis compared to control. There is also enhanced prothrombogenesis in central obesity, suggesting a vital role of waist circumference in obesity-related health risk.

Poster Session

METABOLIC SYNDROME AND SUBCLINICAL ATHEROSCLEROSIS IN YOUNG ADULTS

E.E. Babes, **Victor Vlad Babes**, M.I. Popescu, A. Ardelean

Faculty of Medicine, Oradea, Romania

Background: The metabolic syndrome (MS) is a constellation of interrelated metabolic risk factors that directly promote the development of atherosclerotic cardiovascular disease.

The aim of the study was to investigate the correlation between MS and subclinical atherosclerosis (SA) in a group of young adults.

Methods: We evaluated 102 young healthy adults (age 20-40 y, 58 men) with at least one cardiovascular risk factor. We determined: smoking status, BMI, waist circumference(WC), blood pressure(BP), glucose, lipid profile. SA was assessed with two indicators: endothelial dysfunction evaluated by brachial flow mediated dilation (FMD) and carotid intimae-media-thickness (CIMT). MS was defined according to NCEP ATP III criteria.

Results: MS was present in 17 subjects (16,7%). CIMT values were higher in subjects with MS, 0,54 ±0,03mm vs. 0,48 ±0,08 mm ($p< 0,0001$). In multiple regression analysis independent predictors for CIMT remained: BP ($r=0,48$, $p=0,0002$); HDL cholesterol ($r= - 0,37$, $p=-0,0009$), glucose ($r=0,21$, $p=0,0006$) and WC ($r=0,3$, $p=0,014$). FMD was significantly lower in subjects with MS, 4,25 ±2,32% vs 8,33±3,29% ($p< 0,0001$). In multiple regression analysis independent predictors for FMD were: glucose($r= - 0,19$, $p=0,0094$); HDL-cholesterol ($r= 0,28$, $p=0,007$) and BP ($r= - 0,34$, $p=0,0095$).

Conclusions: MS in young adults is associated with increased prevalence of SA. Endothelial dysfunction is an important arterial abnormality in young subjects with MS. High BP, low HDL-cholesterol and hyperglycemia are especially powerful predictors of increased CIMT and endothelial dysfunction. Central obesity appears to be correlated with CIMT. These findings supports the importance of screening and early intervention in young adults with MS.

Poster Session

ATHEROSCLEROTIC DISEASE LOCATION AND DISPARITIES IN THE CONTROL AND TREATMENT OF CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH TYPE 2 DIABETES

I. Gouni-Berthold[1], K. Bestehorn[2], W. Krone[1], **Heiner K. Berthold**[3]

[1]Department of Internal Medicine II, University of Cologne, Cologne, [2]MSD Sharp & Dohme, Haar, [3]Charite University Medicine Berlin, Berlin, Germany

Aims: To assess differences in the control and intensity of medication treatment of CVD risk factors in patients with type 2 diabetes, depending on their atherosclerotic disease territory (coronary artery disease, [CAD]; cerebrovascular disease, [CBVD]; or peripheral arterial disease, [PAD]).

Methods: Cross-sectional analysis of 17,571 patients with type 2 diabetes with prevalent atherosclerotic disease. Endpoints included uncontrolled CVD risk factors [SBP ≥140 mmHg; LDL-C ≥130 mg/dl; and HbA1c ≥8%] and high intensity of medication treatment (use of two or more classes of antihypertensive agents, one or more lipid-lowering agents and of two or more oral hypoglycemic agents or insulin) in patients with uncontrolled CVD risk factors. Multiple-adjusted ORs were calculated for CAD, CBVD, and PAD after adjusting for sex, age, BMI, current smoking, and diabetes duration.

Results: Proportions of patients with uncontrolled risk factors were significantly different among disease locations. Decreased odds of having lipids not controlled were observed in patients with CAD, while decreased odds of having SBP not controlled were observed in patients with PAD. PAD was associated with the highest odds of hyperglycemia not being controlled. Intensification of treatment was observed in lipid and glycemia management but not in hypertension management independent of disease location. In all disease locations, intensifying antihypertensive medication is worse than intensifying lipid or glycemia treatment.

Conclusions: In subjects with type 2 diabetes and atherosclerotic disease, control of modifiable CVD risk factors but not intensity of medication treatment is modified by atherosclerotic disease territory. Intensity of medication treatment is different between risk factors.

Poster Session

XANTHELASMAS PREDICT RISK OF ISCHEMIC HEART DISEASE, MYOCARDIAL INFARCTION, AND OVERALL DEATH IN THE GENERAL POPULATION

Mette Christoffersen[1], R. Frikke-Schmidt[1], P. Schnohr[2], B.G. Nordestgaard[2,3], A. Tybjærg-Hansen[1,2]

[1]Dept. Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet, [2]The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen, [3]Dept. Clinical Biochemistry, Herlev Hospital, Herlev, Denmark

Introduction: Xanthelasmas are cholesterol deposits of the eyelids. Some similarity has been seen experimentally between the formation of xanthelasmas and the formation of the atherosclerotic plaque, which has led to the question of whether xanthelasmas may be a cutaneous marker of atherosclerosis.

Objective: We examined the hypothesis that presence of xanthelasmas are associated with increased risk of myocardial infarction (MI), ischemic heart disease (IHD), and overall death in the general population.

Methods: A total of 12,939 individuals from the Danish general population, who had presence or absence of xanthelasmas registered at baseline, were followed for up to 33 years; 1,903 individuals developed MI, 3,761 individuals developed IHD, and 8,663 individuals died during follow up. Follow-up was 100% complete. We excluded individuals with IHD diagnosis at baseline.

Results: Xanthelasmas were associated with multivariate adjusted hazard ratios of 1.84 (95% CI, 1.41-2.39) and 1.29 (95% CI, 1.00-1.66) for MI in women and men, respectively. Corresponding hazard ratios for IHD were 1.56 (95% CI, 1.29-1.84) and 1.24 (95% CI, 1.02-1.59). Multivariate adjusted hazard ratios for overall death in individuals with xanthelasmas were 1.18 (95% CI, 1.03-1.35) in women and 1.13 (95% CI, 0.99-1.30) in men.

Conclusion: Presence of xanthelasmas predict risk of MI, IHD, and overall death in individuals initially free of IHD independent of other risk factors including plasma cholesterol levels. Moreover, the risk of IHD and MI is more pronounced in women compared to men.

Poster Session

MIF-DEFICIENCY REDUCES CHRONIC INFLAMMATION IN ADIPOSE TISSUE AND IMPAIRS INSULIN RESISTANCE AND ASSOCIATED ATHEROSCLEROSIS IN A MODEL OF COMBINED DISEASE

Robert Kleemann[1], L. Verschuren[2], P.Y. Wielinga[1], P. Voshol[3], M. van Erk[2], G. Fingerle-Rowson[4], K. Willems van Dijk[5], M. Ouwens[5], J. Bernhagen[6], R. Bucala[7], T. Kooistra[2]

[1]TNO BioSciences, [2]Vascular and Metabolic Diseases, TNO Quality of Life, Leiden, The Netherlands, [3]Cambridge, Cambridge, UK, [4]University of Cologne, Cologne, Germany, [5]LUMC, Leiden, The Netherlands, [6]RWTH Aachen, Aachen, Germany, [7]Yale University School of Medicine, Yale, CT, USA

Chronic inflammation in white adipose tissue (WAT) is positively associated with obesity, insulin resistance (IR) and the development of type-2 diabetes. The pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) is an essential, upstream component of the inflammatory cascade.

We examined whether MIF is required for the development of obesity, IR, glucose intolerance and atherosclerosis in the LDL-receptor-deficient (Ldlr-/-) mouse model of disease. Ldlr-/--mice developed IR and glucose intolerance within 15-w while Mif-/-Ldlr-/- littermates were protected. MIF-deficiency did not affect obesity and lipid risk factors but specifically reduced inflammation in WAT and liver, as reflected by lower plasma SAA and fibrinogen levels at baseline and under inflammatory conditions. Conversely, MIF stimulated the in vivo expression of human-CRP, an inflammation marker and risk factor of IR and cardiovascular-disease. In WAT, MIF-deficiency reduced nuclear c-Jun levels and improved insulin sensitivity; MIF-deficiency also reduced macrophage accumulation in WAT and blunted the expression of proteins that regulate macrophage infiltration (ICAM-1, CD44). Mechanistic parallels to WAT were observed in aorta, where the absence of MIF reduced monocyte adhesion, macrophage lesion content and atherosclerotic lesion size.

Poster Session

THE CLINICAL STUDY OF HEART RATE VARIABILITY AND THE SERIOUS EXTENT OF CORONARY ARTERY LESIONS IN CORONARY HEART DISEASE

Guoping Tian

Department of Cardiology, The Second Affiliated Hospital of Nanhua University, HengYang, China

Objective: To find more diagnostic methods for coronary heart diseases (CHDs), we investigated the relation between them through analysis of heart rate variability and the serious extent of coronary artery lesions in CHDs.

Methods: 120 patients were selected randomly, and were divided into four groups, including stable angina pectoris(SAP) group (n＝30), unstable angina pectoris(UAP) group (n＝30) , ST-segment elevation myocardial infarction(STEMI) group (n=30) and non-ST-segment elevation myocardial infarction (NSTEMI) group (n=30). Besides, there were 31 healthy people as normal control group. All cases were, within 24 hours, checked by dynamic electrocardiogram (DCG). CHDs were checked by coronary arteriography. The results were analyzed with the statistics software of SPSS 13.0.

Results:

1. HRV was low one by one in STEMI group, NSTEMI group, UAP group and SAP group as compared with each other or normal control group, and all of the difference were significant (P< 0.01).

2. With the increase of the severity of coronary artery lesions, the index of HRV which were reflected the function of sympathetic nervous system (SDNN, SDANN, SDNN Index) obviously decreased, but the other index of Heart Rate Variability (HRV)reflected by the function of parasympathetic nervous system (RMSSD, pNN50) were not obviously changed.

Conclusion: HRV can be looked as a no-traumatic index to get the message of the serious extent of coronary artery lesions indirectly.

Keywords: Heart Rate Variability; Coronary Artery Lesion; Coronary Heart Disease.

Poster Session

LDL-CHOLESTEROL AND CARDIOVASCULAR EVENTS IN PATIENTS WITH DIABETES MELLITUS TYPE 2

Constantin Tudor[1], A. Iana[1], R.A. Jeberean[2], A. Petrus[1], R. Buzas[1]

[1]Internal Medicine, [2]Psihiatry, University Hospital Municipal Timisoara, Timisoara, Romania

Background and aims: Type 2 diabetes mellitus (T2DM) is increasingly prevalent worldwide, conferring major burdens on health and health care costs. Development of atherosclerotic cardiovascular disease (CVD) is the principal complication in type 2 DM and a major cause of morbidity and mortality. The aim of the study was to asses the influence of LDL-cholesterol and common carotid intima-media thickness (IMT) on cardiovascular events in patients with T2DM without clinical changes of CVD.

Method and material: The study enrolled 200 patients with T2DM with a mean age of 54.2 ± 7.62 years. T2DM was defined according to WHO criteria. The follow up period was of 3 years. We measured intima-media thickness at the left and right common carotid at 1cm of the bifurcation (average of at least 3 determinations) on B-mode ultrasound images with the use of a 10 MHz linear-array transducer and LDL-cholesterol levels every 6 months.

Results: The characteristics of the studied group at the beginning and end of the study:Table I At start : LDL-cholesterol 102,8±12,8mg/dl, IMT left 10,2±2,3mm , IMT right 9,8±2,5mm After 3 years: 100,2±11,3mg/d, 11,3±2,1mm, 10,2±1,9mm. At 18 patients of the studied group occurred a cardiovascular event during the 3 years of follow up.

Conclusions: Patients with T2DM have modified common carotid intima-media thickness. Cardiovascular events occurred in patients with low levels of LDL-cholesterol. Not only biological parameters are important to follow up in patients with T2DM. Further studies are needed to understand the progression from subclinical to clinical atherosclerotic disease.

Poster Session

THE UCL *LDLR, PCSK9* & *LDLRAP1* VARIANT DATABASES: UPDATE, ANALYSIS & OVERVIEW OF THREE PUBLICLY AVAILABLE FAMILIAL HYPERCHOLESTEROLEMIA GENE DATABASES

Sarah Leigh, T. Jakubcova, R.A. Whittall, S.E. Humphries

Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, University College London, London, UK

Aims: To update and analyse the Low Density Lipoprotein Receptor (*LDLR*) and Pro-Protein Convertase Subtilisin/Kexin Type9 (*PCSK9*) gene variant databases and to establish and analyse a database for Low Density Lipoprotein Receptor Adaptor Protein1 gene (*LDLRAP1*) defects in Autosomal Recessive Hypercholesterolemia (ARH).

Methods: PubMed literature searches were performed and variant data was loaded onto the Leiden Open Source Variant Database platform on the UCL web server. Predicted effects of amino acid substitutions were assessed using the literature and publicly-available computer programs.

Results: The *LDLR* and *PCSK9* databases are available via www.ucl.ac.uk/fh and the *LDLRA1* database will be released in spring 2010. The *LDLR* database lists 1686 variants, representing 1063 unique events. DNA substitutions and small DNA rearrangements continue to represent the majority of variants (65% & 24% respectively); however, a significant number of large rearrangements (~10%) are still reported in *LDLR*, highlighting the importance of screening for such variants. The *PCSK9* database lists 155 variants, representing 101 unique events, *PCSK9* variants appear to be equally divided between loss of function, gain of function or functionally neutral variants. The utility of predictive computer programmes is limited for PCSK9 variants because of their varying effects on cholesterol levels. The *LDLRA1* database will list >20 variants associated with hypercholesterolemia found predominantly in ARH patients.

Conclusion: These three database now list >1100 unique variants associated with aberrant cholesterol levels and provide information relating to pathogenicity, these resources will be of use to researchers and clinicians working in the field of dyslipidaemias.

Poster Session

A MUTATION IN SREBF2 GENE IS INVOLVED IN HYPERCHOLESTEROLEMIA AND HYPERGLYCEMIA

Ana Barbara Garcia Garcia[1,2], S. Martinez-Hervas[1,2,3], S. Vernia[4], C. Ivorra[1], S. Blesa[1,2], J.C. Martin-Escudero[5], J.F. Ascaso[2,3], R. Carmena[2,3], M. Casado[4,6], J.T. Real[2,3], F.J. Chaves[1,2]

[1]Fundación Investigación Hospital Clínico Universitario de Valencia. Instituto Sanitario INCLIVA, Valencia, [2]CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Barcelona, [3]Servicio de Endocrinología y Nutrición, Hospital Clínico Universitario de Valencia, [4]Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, [5]Servicio de Medicina Interna, Hospital Río Hortega, Valladolid, [6]CIBER of Hepatic and Digestive Diseases (CIBEREHD), Barcelona, Spain

Autosomal Dominant Hypercholesterolemias (ADH) are a series of diseases characterised by high total and LDL-cholesterol, which predispose to early atherosclerosis. Mutations in several genes are responsible for ADH: LDLR (Familial Hypercholesterolemia), APOB (Familial Defective ApoB), and PCSK9 (ADH type 3). However, 20-30% of ADH patients do not present mutations in any of these genes. Therefore, other unknown genes must be involved in ADH. SREBP2 is a known transcription factor which regulates several genes involved in lipid metabolism. Our objective has been the analysis of *SREBF2* gene in a sample of 41 ADH patients without mutations in the known genes, searching for alterations that could be responsible for ADH.

We have identified the alteration c.-405A>G, present in patient 104. This mutation was thought to be pathogenic because:

1) It cosegregates with the disease in the family (5 healthy individuals and 7 patients.

2) It has not been found in a sample of 429 healthy controls.

3) Functional studies on cells transfected with plasmids containing the variation have shown that this mutation increases transcription of the gene.

Surprisingly, this mutation also cosegregated with higher glucose levels in the family. An OGTT was performed in 4 family carriers and 28 controls (matched by age, gender and BMI). Carriers presented significantly higher fasting plasma glucose (108±9 mg/dL) than controls (89±32mg/dL), $p \leq 0.05$. To our knowledge, *SREBF2* has not been involved in glucose metabolism in humans. In conclusion mutations in the *SREBF2* gene may result in hereditary hypercholesterolemia (phenotype ADH) and hyperglycemia.

Poster Session

PARAOXONASE-2 GENE (PON2) S311C POLYMORPHISM ASSOCIATED WITH TYPE 2 DIABETIC MELLITUS AND CORONARY HEART DISEASE

Thavatchai Peerapatdit[1], A. Likidlilid[2], N. Hobang[2], D. Tresukosol[1], S. Ratanamaneechat[3]

[1]Medicine, [2]Biochemistry, [3]Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Objective: The variant of the PON2 gene at S311C in exon 9 can lead to dyslipidemia and increase risk for DM, CHD, or both. Therefore, we investigate the associations of S311C polymorphism in type 2 DM with and without CHD compared with healthy control subjects.

Materials and methods: The study included 149 unrelated control subjects, 155 type 2 DM without CHD and 147 type 2 DM with CHD > 50% epicardial stenosis. gDNA was extracted from leukocyte and the variant was determined by PCR-RFLP.

Results: All genotypes of PON2 S311C polymorphism were in consistent with an expected population of Hardy-Weinberg equilibrium. There was a significantly higher frequency of the CC genotype in type 2 DM with and without CHD when compared with control group (p=0.01 and 0.04, respectively). While, C allele frequencies were not different among all three groups. The risks for DM and CHD were assessed and found that the CC genotype appeared to be the risk of both type 2 DM with and without CHD with odds ratio 5.12 (95% CI:1.44-18.22), p=0.01 and 4.08 (95% CI:1.13-14.77), p=0.04, respectively.

Conclusions: There were associations between the PON2 S311C polymorphism and type 2 DM with and without CHD. It was also found that the CC genotype of this polymorphism was significantly higher in type 2 DM with and without CHD than in healthy control. It was possible that the CC genotype can accelerate the development of both type 2 DM and CHD in this population studied.

Poster Session

CAROTID ATHEROSCLEROSIS ASSOCIATED WITH LATE-ONSET DEPRESSIVE DISORDERS: RESULTS FROM A LARGE COHORT STUDY

Adrie Seldenrijk[1], H. van Hout[2], H. van Marwijk[2], E. de Groot[3], J. Gort[3], C. Rustemeijer[4], M. Diamant[5], B. Penninx[1]

[1]Psychiatry, [2]General Practice, VU University Medical Center, [3]Vascular Medicine and Vascular Imaging, Academic Medical Center, University of Amsterdam, [4]Internal Medicine, Amstelland Hospital, [5]Diabetes Center, Internal Medicine, VU University Medical Center, Amsterdam, The Netherlands

Objectives: Carotid intima-media thickness (CIMT) and plaque presence have been established as markers of systemic atherosclerosis. This study examined whether high CIMT and plaque presence were more prevalent among persons with a lifetime diagnosis of depressive or anxiety disorders (n=470) than in controls (n=179), using data from a subcohort (mean age=46 yrs, 65% women) of the Netherlands Study of Depression and Anxiety (NESDA). In addition, the role of disorder characteristics (severity, duration, age of onset) was examined.

Methods: Depressive and anxiety disorders were diagnosed using the DSM-IV based CIDI interview. CIMT and plaque information were obtained using an Acuson Aspen ultrasound instrument with a 5-10MHz broadband transducer. Regression analyses adjusted for sociodemographics, lifestyle and health factors were conducted to investigate the association between psychopathology and carotid atherosclerosis.

Results: Depressive or anxiety disorders were not associated with CIMT or plaque presence. However, of the disorder characteristics, age of onset of depressive disorder was strongly associated with CIMT (0.01 mm per 10 yrs, *p*=.006) and plaque presence (OR=1.34 per 10 yrs, 95%CI=1.02-1.76, *p*=.04). As compared to controls, depressed persons with early onset (< 40 yrs) had no increased plaque presence, but those with late onset (≥40 yrs) had a 1.80 fold increased plaque risk (95%CI=0.87-3.73, *p*=.11).

Conclusion: These findings suggest that a higher atherosclerosis prevalence may be specific for late-onset depressive disorders. This adds to accumulating evidence that late-onset depression, as compared to early-onset depression, has a distinct pathophysiology involving a vascular component.

Supported by NHF grant 2006B258

Poster Session

CARDIOVASCULAR FUNCTION AND PSYCHOLOGICAL DISTRESS IN URBANISED BLACK SOUTH AFRICANS: THE SABPA STUDY

Nyiko Mashele[1], J.M. Van Rooyen[1], L. Malan[1], J.C. Potgieter[2], Hypertension in African Research Team (HART)

[1]Physiology, [2]Psychology, North-West University, Potchefstroom, South Africa

The increased prevalence of cardiovascular disease (CVD) risk factors in Sub-Saharan Africa has increased the prevalence of CVD in this region but whether psychological distress contributes to this observed increased risk remains largely unclear.

Objective: The aim of this study was to investigate the association between psychological distress and CVD risk in urbanised black South African men (n=101) and women (n=99).

Methods: Resting cardiovascular variables were obtained by making use of the Finometer device and 24 hour ambulatory blood pressure measurements with the Cardiotens apparatus. The psychological questionnaires assessed the perception of health (General Health Questionnaire; GHQ-28) and depression status (DSM-IV criteria). The resting ECG (NORAV PC-1200) was used to determine left ventricular hypertrophy (LVH) by making use of the Cornell product. Confounders included age, obesity, alcohol, smoking and physical activity.

Results: The hypertensive group were more overweight with lower vascular compliance and higher LVH (only men) compared to the normotensive groups. In hypertensive men, perception of health (somatic symptoms) was positively associated with blood pressure, while in hypertensive women it was associated with heart rate. Major depression was associated with LVH in hypertensive men and mean arterial pressure in hypertensive women. LVH and depression shoed Odds ratios of 1.02 [(95% CI 0.997-1.05);1.15 (95% CI 1.01-1.32)] respectively, in predicting hypertension in women.

Conclusions: Psychological distress was associated with higher blood pressure in hypertensive African men but also with development of left ventricular hypertrophy in hypertensive African men and women.

Keywords: Depression; perception of health; cardiovascular function; urbanised Africans; hypertension.

Poster Session

FATTY ACIDS IN SERUM PHOSPHOLIPIDS AND CAROTID INTIMA-MEDIA THICKNESS IN SPANISH SUBJECTS WITH PRIMARY DYSLIPIDEMIA

Aleix Sala-Vila[1], M. Cofán[1], A. Pérez-Heras[1], I. Núñez[2], R. Gilabert[2], M. Junyent[1], R. Mateo-Gallego[3], A. Cenarro[3], F. Civeira[3], E. Ros[1]

[1]Lipid Unit; Nutrition and Endocrinology Survey, [2]Diagnosing Imaging Center, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Barcelona, [3]Lipid Unit, Hospital Miguel Servet, Zaragoza, Spain

Introduction: n-3 Long-chain polyunsaturated fatty acids (n-3LC-PUFA) protect from coronary heart disease (CHD). Spain shares with Japan both low fatal and non-fatal CHD rates and high seafood intake.

Objective: To investigate the relationship between n-3LC-PUFA status in serum phospholipids as biomarkers of seafood intake and carotid atherosclerosis in Spanish subjects at risk for CHD.

Design: In a cross-sectional study, 451 asymptomatic subjects (261 men, 190 women, mean age 45 years) with primary dyslipidemia had fatty acids measured in serum phosphatidylcholine and carotid intima-media thickness (IMT) determined by ultrasound. Fatty food and seafood intake was evaluated in a random subsample of 70 participants.

Results: Phosphatidylcholine fatty acid composition and mean daily consumption of olive oil (39 g) and seafood (76 g) were similar to those reported for Spanish populations. Multiple regression analyses showed that eicosapentaenoic + docosahexaenoic acids (β = -0.094, P = 0.015) and oleic acid (β = -0.107, P = 0.005) proportions were inversely related to mean common carotid artery IMT after adjustment for cardiovascular risk factors, statin use and other fatty acids. In similar models, α-linolenic acid related inversely to bifurcation and internal carotid artery (ICA)-IMT, while linoleic acid was directly related to ICA-IMT ($P < 0.05$, all).

Conclusion: Increasing phospholipid proportions of n-3LC-PUFA, oleic acid and α-linolenic acid are associated with less atherosclerosis in subjects with primary dyslipidemia. High intakes of seafood and olive oil might explain in part the Spanish paradox of low CHD rates in face of a high burden of cardiovascular risk factors.

Poster Session

RECLASSIFICIATION OF 10 YEAR CHD RISK COMPARED BY PRESENCE OF CAROTID PLAQUE AND HIGH CRP

Vilmundur Gudnason[1,2], S. Halldorsdottir[1], B. Thorsson[1], T. Aspelund[1,2]

[1]Icelandic Heart Association Research Institute, Kopavogur, [2]University of Iceland, Reykjavik, Iceland

Introduction: Carotid plaque and CIMT are used as surrogate markers for CAD. The REFINE Reykjavik Study is a population based study where the objective is to refine cardiovascular risk evaluation.

Aims: To compare reclassificiation within categories of 10 year Reykjavik study CHD risk using presence of carotid plaque and high CRP levels.

Methods: Since 2006, 4025 subjects (51% women) aged 35-69 have been recruited from a random sample stratified by sex and age from the greater Reykjavik Area, with a response rate of 70%. Carotid plaque and CIMT were measured using ultrasound. At least moderate plaque defined the presence of plaque and high CIMT >75%. 3437 apparently healthy men without CHD history or statin use were analysed for10-year CHD risk based on classical risk factor levels and then reclassified by presence of carotid plaque and having high CRP (≥3 mg/L). All results were population weighted.

Results: The table shows estimated prevalence of plaque, CIMT, high CRP, and combinations of conditions in men. Results were comparable for women.

10-year CHD risk categories	0-3%	3-6%	6-10%	10+ %	Total
Plaque	1.2%	4.9%	10.1%	25.3%	8.4%
Plaque or high IMT(>75%)	7.8%	21.0%	45.7%	66.9%	29.6%
CRP≥3 mg/L	10.3%	17.0%	19.6%	28.9%	17.3%
CRP≥3 mg/L AND plaque	0.2%	1.1%	1.5%	9.2%	2.3%
CRP≥3 mg/L AND (Plaque or high IMT)	1.1%	4.2%	9.9%	21.3%	7.4%

[Reclassification of CHD risk in men]

Conclusion: High CRP is frequent in all risk categories, but has little overlap with evidence of carotid artherosclerosis in either men or women.

Poster Session

INSTABILITY OF CORONARY PLAQUES IS CLINICALLY AND ANGIOGRAPHICALLY ASSOCIATED WITH SPECIFIC CRP-RECEPTOR SUBTYPE

Dorette Raaz-Schrauder[1], L. Klinghammer[1], A.B. Ekici[2], M. Herrmann[3], W.G. Daniel[1], A. Reis[2], C.D. Garlichs[1]

[1]Cardiology and Angiology, [2]Institute of Human Genetics, [3]Medical Clinic III, University Hospital Erlangen, Erlangen, Germany

Purpose: Clinical outcome in patients with acute coronary syndromes (ACS) is strongly influenced by inflammation and its central mediator C-reactive protein (CRP). Recently, Fcχ receptor IIa (FcχRIIa) has been identified as the receptor for CRP. The aim of our study was to assess whether patients with ACS show a specific genetic subtype for FcχRIIa (i.e. the allele H131 with weak CRP binding or R131 with strong CRP binding). Additionally, analyses of patients' coronary angiograms evaluated the association of FcχRIIa genetic subtype with appearance of intracoronary thrombus.

Methods: We conducted a genetic association study among 701 consecutive patients with first event of ACS compared to 467 patients with stable angina pectoris. All patients were genotyped for a frequent functional polymorphism of the mature FcχRIIa. Moreover, angiographic analyses classified patients' angiograms according to the type and grade of stenoses as well as the presense of intracoronary thrombus.

Results: The FcχRIIa R/R131 genotype was significantly associated with ACS as the first manifestation of CAD (P=1.2 x 10-9, odds ratio 2.86, 95% CI:2.06-3.99) compared to the non-R/R131 genotype. Coronary angiograms of ACS patients with the FcχRIIa R/R131 genotype showed significantly higher frequencies of intracoronary thrombus and more complex lesions as compared to ACS patients with non-R/R131.

Conclusions: Our data show a genetic association of the FcχRIIa R/R131 genotype with a more frequent occurrence of ACS as the first manifestation of CAD. Moreover, coronary lesions of ACS patients with this genotype show significantly higher complexity and frequently contain thrombus.

Poster Session

ENDOTHELIAL FUNCTION ASSESSED BY PERIPHERAL ARTERY TONOMETRY IS A DETERMINANT OF CAROTID INTIMAE-MEDIA THICKNESS REGARDLESS OF FRAMINGHAM RISK SCORE

Raimon Ferré[1], G. Aragonès[2], N. Plana[1], J. Merino[2], M. Heras[2], B. Coll[3], R. Cos[2], L. Masana[1]

[1]Internal Medicine. Unitat de Medicina Vascular i Metabolisme, Hospital Sant Joan. Universitat Rovira i Virgili. CIBERDEM., [2]Unitat de Recerca de Lípids, Universitat Rovira i Virgili, CIBERDEM, Reus, [3]Hospital Arnau de Vilanova, Lleida, Spain

Peripheral artery tonometry (PAT) seems to be a reliable clinical method to assess endothelial function (EF). Its correlation with carotid intimae-media thickness (cIMT) in patients with intermediate cardiovascular (CV) risk as assessed by the Framingham Risk Score (FRS) has not been established.

Objective: To assess EF using PAT in patients with intermediate CV risk by FRS and determine its association with cIMT.

Design: We studied 248 patients with intermediate CV risk and no history of cardiovascular disease. EF by PAT, cIMT and a physical exam, as well as anthropometrical and biochemical studies were performed.

Results: EF assessed by PAT, along with LDL and age, was the main predictor of increased cIMT. PAT values were independent of FRS. PAT was lower in men than women (P=0.025). Smokers also had a lower PAT (P=0.004). In a bivariate correlation analysis, the PAT ratio was inversely associated with waist circumference (WC) and triglycerides and directly associated with HDL and Apo A1. PAT was also positively correlated with age and systolic blood pressure (SBP) (P=0.004 and P=0.003, respectively). In a linear regression model, the factors associated with a lower PAT ratio were smoking, WC, triglycerides and diabetes therapy, while lipid-lowering therapy and pulse pressure were positively correlated.

Conclusions: Endothelial dysfunction assessed by PAT is a determinant of cIMT in patients at intermediate cardiovascular risk. A lower PAT is associated with the lipid and obesity components of the metabolic syndrome. Since PAT is not correlated with FRS, this measure of EF may provide clinical information beyond classical cardiovascular risk scores.

Poster Session

OPTIMISING THE SELECTION OF GENETIC MARKERS FOR MENDELIAN RANDOMISATION EXPERIMENTS

Daniel Swerdlow[1], R. Sofat[2], T. Shah[2], K. Kuchenbaecker[1], J. Mindell[1], M. Kumari[1], M. Kivimaki[1], J.P. Casas[3], E. Brunner[1], A. Hingorani[1,2]

[1]Department of Epidemiology & Public Health, [2]Centre for Clinical Pharmacology, Department of Medicine, University College London, [3]Department of Epidemiology & Population Health, London School of Hygiene and Tropical Medicine, London, UK

Background: Through Mendelian randomisation, association between a biomarker and a disease is interpreted as causal if genetic variants affecting the level of that biomarker are also associated with the disease. However, careful consideration is needed to select appropriate genetic variants. Protein biomarkers e.g. C-reactive protein (CRP), teleologically have a single, proximal associated gene, whereas non-protein biomarkers e.g. HDL-cholesterol, are influenced by multiple genes which may also affect other biomarkers.

Objectives: To examine the specificity of phenotypic effects of common genetic variants associated with CRP and HDL-cholesterol.

Methods: We assessed CRP, HDL-cholesterol and 28 other biomarkers and selected single nucleotide polymorphisms (SNPs) most closely associated with CRP (10 SNPs) and HDL-cholesterol (7 SNPs) from the IBC HumanCVD BeadChip genotyped in 5060 men and women from the Whitehall-II study.

Results: The SNP (rs1205) in *CRP* (1q21) most closely associated with CRP (p=1.85x10^{-8}) affected only CRP concentration, but SNPs in other genes associated with CRP [e.g. the *APOE* cluster (19q13) and *IL6R* (1q21)] additionally affected LDL-cholesterol and interleukin-6, among other traits. A SNP (rs17231506) in *CETP* (16q13) was most strongly related to HDL-cholesterol (p=2.49x10^{-15}) but also affected LDL-cholesterol, and apolipoproteins A1 and B. Multiple effects were also found for other HDL-cholesterol-related SNPs [including rs301 *LPL* (8p21), rs231342 *LIPC* (15q22), rs662799 *APOA5* cluster (11q23)].

Conclusions: Care must be taken in selecting genetic markers for Mendelian randomisation experiments to avoid potential confounding by pleiotropic effects. A specific genetic proxy is likely to be found for a protein biomarker but not necessarily for non-protein traits.

Poster Session

INTERACTION OF SELECTED ABCG1 GENE SINGLE NUCLEOTIDE POLYMORPHISMS WITH DIETARY PUFAS TO MODULATE HDL-CHOLESTEROL CONCENTRATIONS IN THE POPULATION-BASED HORTEGA STUDY

Rosario Abellan[1], M.L. Mansego[1,2], S. Martínez-Hervás[1,3,4], J.C. Martín-Escudero[5], R. Carmena[3,4], J.T. Real[3,4], J. Redon[2,6], M. Pineda[5], F.J. Chaves[1,4]

[1]Genotyping Unit and Genetic Diagnostic, Fundación Investigación Clínico de Valencia, Instituto de Investigación Sanitaria INCLIVA, [2]CIBER de Obesidad y Nutrición "CIBEROBN", [3]Servicio de Endocrinología, Hospital Clínico Universitario de Valencia, [4]CIBER de Diabetes y Enfermedades Metabólicas Asociadas "CIBERDEM", Valencia, [5]Servicio de Medicina Interna, Hospital Rio Hortega, Valladolid, [6]Servicio de Medicina Interna, Hospital Clínico Universitario de Valencia, Valencia, Spain

Introduction: Although previous studies have observed that ABCG1 mediates cholesterol transport from macrophages to the major HDL fractions, there is little information about ABCG1 polymorphisms and variations in plasma HDL-cholesterol concentrations in humans.

Objective: The aim of our study was to investigate whether interactions between polyunsaturated fatty acids intake and SNPs located within the ABCG1 gene modulate the observed associations with HDL-cholesterol concentrations under postprandial state in a representative Spanish Caucasian population.

Design: ABCG1 SNPs were genotyped in 1270 subjects (50.0% women, age range 21-85 years old) who participated in the population-based Hortega study. Biochemical measurements were made by using standard procedures. Dietary intakes were estimated by using a validated questionnaire.

Results: Significant gene-diet interactions for HDL-cholesterol concentrations were found (P interaction=0.0004), although no significant differences in dietary intake were observed according to genotype groups. For the SNP rs1044317 (c.*399 A>G, located in the 3' UTR), homozygotes for the G allele had higher HDL-cholesterol concentrations than did carriers of the A allele under diets with a low polyunsaturated fatty acids content (P=0.043). However, under diets with a high polyunsaturated fatty acids content, the G allele homozygotes had significant lower HDL-cholesterol concentrations than did carriers of the A allele (P=0.003).

Conclusions: In a representative Spanish population under postprandial state, the ABCG1 rs1044317 polymorphism was associated with variations in plasma HDL-cholesterol concentrations, mainly in subjects with high polyunsaturated fatty acids intakes.

Poster Session

***MRAS* - A STRONG CANDIDATE GENE (SCG) FOR CORONARY ARTERY DISEASE (CAD)**

Jennifer Freyer[1], A.K. Wagner[2], H. Schunkert[1], J.-J. Zwaginga[3], Z. Aherrahrou[1], P. Linsel-Nitschke[1], J. Erdmann[1]

[1]Medizinische Klinik II, Universität zu Lübeck, Lübeck, Germany, [2]Karolinska Institut, University Stockholm, Stockholm, Sweden, [3]Leiden University Medical Center, Leiden, The Netherlands

In our recent genome-wide association study (GWAS) we identified the *MRas* gene as a SCG for CAD. M-Ras is a member of the Ras superfamily of small GTPases, thes family members function as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes. Atherosclerosis is a chronic inflammatory disease of the vessel wall with accumulation of lipid-laden macrophages in the large arteries. RT-PCR analyses demonstrated that *MRas* is expressed in several tissues, including mouse and human aorta and heart, tissues that are involved in atherosclerosis. M-Ras has been implicated in the regulation of TNFα-stimulated LFA-1 activation and integrin-mediated leukocyte adhesion downstream of various inflammatory cytokines. M-Ras could thus be linked to monocyte adhesion onto the endothelium. We have obtained *MRas*-KO mice and we are currently crossing *MRas*-null mice with Apolipoprotein E (ApoE)-KO mice in order to create double-KO animals because *Apoe*-KO mice are highly susceptible to atherosclerosis. We will perform a mouse-atherosclerosis study.

Poster Session

MODEL OF TYPE 2 DIABETES COMBINED WITH HYPERCHOLESTEROLEMIA INDUCED BY DIET PROMOTES TARGET ORGAN LESIONS IN NEW ZEALAND RABBITS

Tatiana Helfenstein[1], F. Fonseca[1], S. Ihara[2], J. Bottos[3], M. Farah[3], F. Moreira[1], D. Pomaro[2], H. Pott Jr[4], L. Godoy[1], R. Povoa[1], A. Carvalho[1], M. Izar[1]

[1]Medicine, [2]Pathology, [3]Ophtalmology, Federal University of Sao Paulo, Sao Paulo, [4]Medicine, Pontificy Catholic University of Campinas, Campinas, Brazil

Objectives: The aims of this study were to develop an experimental model of type 2 diabetes mellitus combined with hypercholesterolemia induced by diet and to assess glucometabolic alterations and target organ lesions.

Methods: New Zealand male rabbits were fed high-fat/high-sucrose (10/40%) and cholesterol-enriched diet for 24 weeks, when they were sacrificed. Biochemistry, fundus photographs with fluorescein angiography, and pathological analyses were performed.

Results: The animals gained weight, increased blood glucose, total cholesterol, LDL-C and triglycerides, and decreased HDL-C ($p< 0.05$ vs. baseline). Fructosamine levels and the homeostasis model assessment of insulin resistance (HOMA-IR) were increased, while there was a reduction in the HOMA-β ($p< 0.05$ vs. baseline). Early clinical features of diabetic retinopathy, with hyperfluorescent dots consistent with presence of retina microaneurysms were seen since week 12, progressing up to the end of the experiment ($p< 0.0005$). Aortic atherosclerosis, hepatic steatofibrosis, glomerular macrophage infiltration and foci of interstitial fibrosis in the kidneys were the main histomorphologic findings of this study.

Conclusions: Our model reproduced several glucometabolic characteristics of humanoid type 2 diabetes and promoted target organ lesions and atherosclerosis. This non-expensive model is suitable for studying mechanistic pathways and allowing novel strategic approaches.

Poster Session

INCREASED ERYTHROCYTE AGGREGABILITY IN PATIENTS WITH SLOW CORONARY FLOW

Kumral Ergun Cagli[1], E.I. Gurel[2], O. Ozeke[3], N. Seringec[2], A. Yalcinkaya[4], S. Kocabeyoglu[4], F.N. Basar[1], N. Sen[1], K. Cagli[4], N. Dikmenoglu[2]

[1]Cardiology, Turkiye Yuksek Ihtisas Hospital, [2]Physiology, Hacettepe University, Faculty of Medicine, [3]Cardiology, Special Akay Hospital, [4]Cardiovascular Surgery, Turkiye Yuksek Ihtisas Hospital, Ankara, Turkey

Objective: Slow coronary flow (SCF) is a well-known entity, but its exact pathophysiology is still unclear. Microvascular dysfunction is typically implicated in the pathogenesis of SCF, but less attention has been paid to intrinsic properties of blood that can also impair the microcirculatory blood flow. In the present study we aimed to evaluate aggregability and deformability of erythrocytes and viscosity of plasma in SCF.

Methods: Twenty-three patients with SCF (Study group, 15 male, mean age 55±13.8years) and 20 subjects with normal coronary arteries (Control group, 12 male, mean age 58.5±10.7years) were included in the study. Coronary blood flow was quantified by means of thrombolysis in myocardial infarction (TIMI) frame count and aggregation and deformability of erythrocytes were measured by Laser-assisted Optical Rotational Cell Analyser. Plasma viscosity was measured by a cone-plate viscometer. Erythrocyte aggregation was represented by aggregation half-time, aggregation amplitude, and aggregation index, whereas elongation index was used for expression of deformability.

Results: Aggregation half-time, aggregation index, elongation index, and plasma viscosity were similar between two groups, however, significant difference was found in aggregation amplitude, which was in favour of an increased aggregability in SCF patients compared to controls (22.5±4.7au vs. 17.5±7.5au, respectively, p=0.028). In the study group, correlation analysis revealed a significant relationship between the TIMI frame count for left anterior descending artery and aggregation amplitude (r=0.307, p=0.045).

Conclusion: These findings suggest that erythrocyte aggregability, but not deformability or plasma viscosity may contribute to the pathophysiology of SCF presumably via adversely affecting blood flow in the coronary microcirculation.

Poster Session

INVOLVEMENT OF NA+ -CA2+ EXCHANGER IN THE ENDOTHELIUM-INDEPENDENT VASORELAXATION INDUCED BY CURCUMA LONGA L IN RAT MESENTERIC ARTERY

Oluwatosin Adaramoye[1], I.A. Medeiros[2]

[1]Biochemistry, University of Ibadan, Ibadan, Nigeria, [2]LTF, Universidade Federal da Paraíba (UFPB), Joao Pessoa, Brazil

Objective: Previous studies confirmed that the methanolic extract from Curcuma longa L (CLME) lowers arterial blood pressure and heart rate in rats. In this study, we investigated the involvement of Na+ -Ca2+ exchanger (NCE) in the vasorelaxation by CLME in rat mesenteric arteries.

Methods: Mesenteric arteries were obtained from rats and cut into rings. In some rings, the endothelium was removed by gently rubbing the surface with a moist finger. The rings were suspended in organ baths containing Tyrode´s solution (37oC, 95% O2 + 5% CO2). Rings were stabilized with a resting tension of 0.75 g for 60 minutes, and different drugs were administered.

Results: CLME (1-1000 µg/mL) concentration-dependently relaxed phenylephrine (PHE) (10 µM) pre-contracted rings with intact-or denuded-endothelium, suggesting that the removal of endothelium has no effect on vasorelaxation by CLME. CLME (30, 100 and 300 µg/mL) inhibited the cumulative concentration-response curves to PHE (10-8 -10-5 M) in a concentration-dependent manner. Treatment with ouabain 100 µM (selective blocker of Na+-K+ ATPase) has no effect on the relaxant responses of CLME. However, treatment with nickel chloride (NiCl2) (100, 300 and 400 µM), a putative NCE inhibitor, concentration-dependently reduced the vasorelaxant responses of CLME. CLME (100 µg/mL) produced less relaxant effect with decreasing extracellular Na+ concentration. CLME-induced vasorelaxation was abolished in a Na+ -free Tyrode´s solution, a condition that eliminates the influence of the forward mode of the exchanger.

Conclusions: The results provide indirect evidence that the stimulation of the forward mode of NCE may contribute to the vasorelaxation by CLME.

Poster Session

BODY MASS INDEX IS A MAJOR DETERMINANT FOR NON-ALCOHOLIC STEATOHEPATITIS IN NON-DIABETIC HYPERTENSION PATIENTS

Chang-Hua Chou[1], W.-C. Tsai[2]

[1]Internal Medicine, Sinlau Christian Hospital, [2]Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan R.O.C.

Purpose: Non-alcoholic steatohepatitis (NASH) was associated with cardiovascular risk. Metabolic syndrome was associated with NASH. However, factors for development of NASH were not well study in hypertension.

Methods: We included 220 non-diabetic essential hypertension patient (mean age 41 ± 8 years, 149 men) in this study. All patients did not have viral or alcoholic liver diseases. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and adiponectin were measured. NASH was defined as if ALT or AST >30 U/L in men or >19 U/L in women. Homeostasis model assessment (HOMA) index was used for degree of insulin resistance.

Results: NASH was noted in 99 patients (45%). Patients with NASH had significantly higher glucose, triglyceride, cholesterol, body mass index (BMI), HOMA index, and lower adiponectin. Status of statin usage was not associated with NASH. After multivariate analysis controlling glucose, triglyceride, cholesterol, BMI, HOMA index, and adiponectin, only BMI was significantly associated with NASH (HR 1.179, 95% CI 1.059-1.313, p = 0.003), and cholesterol was borderline significantly associated with NASH (HR 1.010, 95%CI 1.000-1.019, p = 0.053).

Conclusion: BMI is an independent factor associated with NASH in hypertension.

Poster Session

INCREASED LEVELS OF ADMA AS MEASURE OF ENDOTHELIAL DYSFUNCTION IN ELDERLY PATIENTS WITH ATRIAL FIBRILLATION

Ingebjørg Seljeflot[1], S. Ulimoen[2], S. Enger[2], H. Arnesen[1], A. Tveit[2]

[1]Center for Clinical Heart Research, Dept of Cardiology, Oslo University Hospital, Ullevål, Oslo, [2]Medicine, Asker & Bærum Hospital, Sandvika, Norway

Several independent risk factors for AF have been identified, however, the importance of endothelial dysfunction is still not clarified.

The aim of the present study was to evaluate the levels of L-arginine, the substrate for nitric oxide (NO) and the asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO-synthase, as related to the presence of AF.

Material and methods: The study population consists of 75-year old subjects with permanent AF (n=62) and control subjects in sinus rhythm (n=124), matched for gender. Clinical data were obtained and fasting blood samples were prepared for measurement of L-arginine and ADMA, performed by an HPLC-method. Group differences were compared by t-test and Chi-square was used for trend analysis through quartiles. Multiple regression models were performed for estimation of independency.

Results: Means (SD) are given. Levels of ADMA were elevated in AF vs controls (0.69 (0.13) vs 0.62 (0.12) umol/L, p< 0.001) and the L-arginine/ADMA ratios were lower (114 (23) vs 124 (27), p=0.015), still significant after adjustment for relevant covariates (creatinine, hypertension, body mass index, diabetes, ischemic heart disease, LDL-cholesterol) (p=0.007 and p=0.037, respectively). When dividing the ADMA levels into quartiles there was a significant trend for having AF with increasing levels of ADMA (p=0.001) with a clear cut-off at the 25th percentile (< 0.54umol/L), giving an OR for having AF of 7.16 (95% CI 2.43-21.09) with higher levels.

Conclusion: Elevated levels of ADMA are significantly predicting the presence of atrial fibrillation in the elderly, elucidating the importance of endothelial dysfunction in such patients.

Poster Session

AORTIC STIFFNESS AS A RISK FACTOR FOR RECURRENT CARDIOVASCULAR EVENTS IN VERY HIGH RISK DIABETIC MEN

Dimitrios Levisianou[1], A. Melidonis[1], S. Foussas[1], E. Adamopoulou[1], E. Skopelitis[2], A. Destounis[2], T. Xenopoulou[2], I. Skoularigis[3], G. Koukoulis[3], F. Triposkiadis[3]

[1]Tzanio General Hospital, Piraeus, [2]Agios Panteleimon General Hospital, Nikea, [3]University of Thessaly, Larissa, Greece

Background and aims: Aortic stiffness (AS) has been shown to correlate with the possibility of recurrence of coronary events in non-diabetic patients. Its predictive value for cardiovascular morbidity in diabetics with ischaemic heard disease isn't established. The aim of this prospective study was to test the hypothesis that estimating AS could predict cardiovascular morbidity in diabetic patients.

Materials and methods: One hundred consecutive diabetic men with no history of cardiovascular disease, admitted to the coronary care unit, were evaluated for arterial stiffness by calculating carotid-femoral pulse wave velocity (PWV). Follow-up telephone interviews at 12 months after discharge assessed the primary endpoints: unstable angina, revascularization, myocardial infarction, stroke or death. Cox proportional hazard models were used to estimate cause-specific hazards of the end-points. To facilitate analysis, PWV values were divided into two halves, upper and lower.

Results: Overall 12 cardiovascular events and 2 deaths were recorded during follow-up period. Those in the upper compared to those in the lower half of PWV were older (68.22±7.18 vs. 58.96±10.36 years, p< 0.001), had a longer diabetes duration (13.6±10.43 vs. 6.71±5.95 years, p=0.003), and more increased TC (178.26±52.50 vs. 216.66±56.7 mg/dl, p=0.025). After adjustment for age, diabetes duration, TC, and smoking, PWV was independently associated with the risk for recurrent cardiovascular events (hazard ratio=2.408, 95%CI=1.313-6.88, p=0.032).

Conclusion: Increased AS was associated with increased one-year risk for recurrent cardiovascular event in diabetic men. We propose that AS could be routinely used for risk stratification in the management of diabetic men after the first coronary event.

Poster Session

GENETIC VARIATION AT THE *CD36* LOCUS AND RISK OF CORONARY HEART DISEASE IN WOMEN AND MEN

Majken K Jensen[1], K. J Mukamal[2], C. J Girman[3], C. Chen[1], A. Handberg[4], K. M Rexrode[5], E. B Rimm[1]

[1]Harvard School of Public Health, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]Merck Research Labs, Noth Wales, PA, USA, [4]Aarhus University Hospital, Aarhus, Denmark, [5]Brigham and Women's Hospital, Boston, MA, USA

Introduction: CD36 is a scavenger receptor that is important for the differentiation of macrophages into foam cells. Genetic variation at this locus has been associated with lipids and risk of CAD, especially among men.

Aim: We examined whether genetic variation at this locus was associated with risk of CHD in two prospective studies of women and men.

Methods: We genotyped 28 SNPs spanning 77 kbp at the *CD36* region on Chr 7 in two nested case-control studies within the Nurses' Health and Health Professionals Follow-Up Studies (967 cases and 1929 controls).

Results: We identified two major haplotype blocks of high linkage disequilibrium that were tagged by four common htSNPs (rs#´s: 9641866, 1953298, 3211886, 3211931). When considered together, five common haplotypes were estimated (frequency >= 5%). Compared to the haplotype carrying all major alleles, we observed a 30% higher risk of CHD for a haplotype that included variant alleles for rs1953298 and rs3211886. Upon separate analyses of the htSNPs, only the rs3211886 variant was associated with risk of CHD, especially in men (p, interaction with gender=0.05). Further stratification of the female cohort suggested that the haplotype was associated with CHD in postmenopausal women who did not use hormonal therapy (OR=1.8, 95% CI: 1.1-3.0) whereas no risk was observed in postmenopausal women with exogenous hormone use or in premenopausal women (OR=1.1, 95% CI: 0.6-1.9).

Conclusion: This study suggests that common genetic variation at the *CD36* locus may predict risk of CHD - especially among men and women with low estrogen levels.

Poster Session

HETEROZYGOSITY FOR R1141X IN *ABCC6* AND RISK OF ISCHEMIC VASCULAR DISEASE

Louise S. Hornstrup[1], A. Tybjærg-Hansen[1], C. Lundegaard Haase[1], B.G. Nordestgaard[2], P. Grande[3], R. Frikke-Schmidt[1]

[1]Dept. Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, [2]Dept. Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, [3]Dept. Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Background and aims*:* Pseudoxathoma elasticum (PXE) is an autosomal recessive disease caused by mutations in *ABCC6.* The disease leads to dystrophic mineralization in elastic components of skin, eyes and vessels, the latter leading to an increased risk of ischemic cardiovascular disease in PXE patients. Previous research has suggested that heterozygotes may display an attenuated disease phenotype. Thus, we tested the hypothesis that heterozygosity for R1141X, the most frequent PXE-causing mutation in Caucasians, leads to an increased risk of ischemic vascular disease in the general population.

Patients and methods*:* We genotyped 10,276 individuals in the Copenhagen City Heart Study; 51,135 individuals in the Copenhagen General Population Study; 4,859 individuals in the Copenhagen Ischemic Heart Disease Study and 640 individuals in the Copenhagen Carotid Stroke Study for the R1141X mutation.

Results*:* The frequency of R1141X was 0.6% in all the populations studied. We observed no increased risk of ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease or ischemic stroke in either of the cohorts. Furthermore, we found no association between R1141X and any ischemic event in an analysis of all studies combined, with a total of 66,910 individuals and 13,668 ischemic events.

Conclusion*:* In four large studies, comprising a total of 66,910 participants of which 13,668 had ischemic events; R1141X did not associate with risk of ischemic vascular disease. Thus, heterozygosity for the R1141X mutation does not seem to cause a moderate PXE phenotype in the general population.

Poster Session

TCF7L2: A GENETIC LINK BETWEEN DIABETOGENICITY AND ATHEROGENICITY?

Axel Muendlein[1,2], C.H. Saely[1,2,3], S. Geller-Rhomberg[1], G. Sonderegger[1], P. Rein[1,2,3], T. Winder[1,2,3], S. Beer[1,2,3], J. Breuss[1], A. Vonbank[1,2,3], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria, [2]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [3]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Coronary artery disease (CAD) shares common risk factors with type 2 diabetes (T2DM). Variations in the transcription factor 7-like 2 (TCF7L2) gene, particularly rs7903146, increase T2DM risk. Potential links between TCF7L2 variant rs7903146 and coronary atherosclerosis are unknown. We therefore investigated the association between rs7903146 and angiographically determined CAD in diabetic and non-diabetic patients.

Methods and results: We genotyped variant rs7903146 in a large cohort of 1647 consecutive Caucasian patients undergoing coronary angiography for the evaluation of established or suspected stable CAD. Significant CAD was diagnosed in the presence of coronary stenoses ≥50%. Variant rs7903146 in the total study cohort was significantly associated with significant CAD (adjusted additive odds ratio (OR) 1.29 [1.09-1.53]; p=0.003). This association was strong and significant in T2DM patients (n=391; OR=1.90 [1.32-2.74]; p=0.001) but not in non-diabetic subjects (OR=1.10 [0.90-1.34]; p=0.370). The interaction risk allele by T2DM was significant ($p_{interaction}$=0.002), indicating a significantly stronger impact of the polymorphism on CAD in T2DM patients than in non-diabetic subjects. Further, the association of variant rs7903146 with T2DM depended on the presence of CAD: The polymorphism strongly predicted T2DM in patients with significant CAD (OR=1.57 [1.26-1.97]; p< 0.001) but not in subjects without significant CAD (OR=1.04 [0.77-1.40]; p=0.791; $p_{interaction}$=0.019).

Conclusions: TCF7L2 variant rs7903146 is significantly associated with angiographically diagnosed CAD. This association is significantly modulated by the presence of T2DM; conversely the impact of rs7903146 on T2DM risk is significantly modulated by the presence of CAD. These data point to TCFL2 as genetic link between atherogenesis and diabetogenesis.

Poster Session

CORONARY ARTERY DISEASE GENOME-WIDE REPLICATION AND META-ANALYSIS (CARDIOGRAM) - DESIGN OF A PROSPECTIVE META-ANALYSIS OF 14 GENOME-WIDE ASSOCIATION STUDIES

Jeanette Erdmann, CARDIoGRAM

University of Luebeck, Lübeck, Germany

GWAS have uncovered at least 13 common alleles associated with CAD. However, each variant confers a modest effect, and together, the variants explain a small fraction of heritability. These observations suggest that additional loci harboring CAD-associated variants might be discoverable with larger samples and improved statistical power. To accomplish this, we assembled CARDIoGRAM that pools GWAS data from 14 studies. In total, the consortium comprises more than 22,000 cases with CAD and more than 60,000 controls. In each individual study, genome-wide genotyping was carried out, and imputation was conducted to generate genotypes for 2.2 million SNPs in each study.

With the aim of conducting a type II meta-analysis using GWA results from the single studies and consortia, SOPs were generated in order to harmonize the QC and data analyses. With the assembled sample size, the estimated power to detect modest effects is substantially increased. For instance, even for genome-wide significance, the power is about 80% for an odds ratio of 1.1, provided that the minor allele frequency is at least 10%.

Meta-analyses for CAD phenotype as well as for important subgroups including MI and early-onset CAD will be carried out. Following the initial evaluations, wet lab replication genotyping of top results will be sought in more than 15,000 additional cases and 15,000 controls. CARDIoGRAM brings together an enormous wealth of GWA studies data on CAD and myocardial infarction, thus representing the largest study to date to uncover the inherited basis for the leading public health problem in the industrialized world.

Poster Session

IDENTIFICATION AND FUNCTIONAL ANALYSES OF MOLECULAR HAPLOTYPES OF THE HUMAN OSTEOPROTEGERIN GENE PROMOTER

C. Hagedorn[1], **Ralph Telgmann**[2], C. Dördelmann[3], B. Schmitz[2], S. Hasenkamp[4], F. Cambien[5], M. Paul[6], E. Brand[7], S.-M. Brand-Herrmann[2]

[1]University Witten/Herdecke, Witten, [2]Molecular Genetics of Cardiovascular Disease, Leibniz-Institute for Arteriosclerosis Research, Münster, [3]Institute for Analytical Sciences, Dortmund, Germany, [4]University of Gothenburg, Gothenburg, Sweden, [5]INSERM UMRS 937, Paris, France, [6]Maastricht University, Maastricht, The Netherlands, [7]University Clinic Münster, Münster, Germany

Osteoprotegerin (OPG) has been reported to be involved in the development of atherosclerotic disease phenotypes, and OPG gene variation has been associated with plasma OPG levels and CAD phenotypes. The architecture of the *OPG* promoter and its transcriptional regulation are poorly characterized. We identified 1008 bp of the *OPG* 5'-flanking region to be sufficiently active in osteosarcoma cells. To identify *cis*-regulatory regions, serial deletion constructs were generated. Variants T-960C, A-946G, G-900A, T-864G, and T-159C within the 5'-flanking region were identified, and individual subcloning revealed the existence of three molecular haplotypes (MolHaps): [T^{-960}-A^{-946}-G^{-900}-T^{-864}; MolHap1, wild type (wt)], [T^{-960}-G^{-946}-G^{-900}-T^{-864}; MolHap2], [C^{-960}-G^{-946}-A^{-900}-G^{-864}; MolHap4]. The *OPG* full length construct (1008 bp) was sufficiently, transcriptionally active, whereas activities of MolHaps 2 and 4 were significantly reduced (p = 0.0018). Introduction of the -159C allele reduced transcriptional activities of the respective full length constructs (p = 0.0014), and significantly increased activities of the deletion constructs (p = 0.0005). EMSAs, competition assays, and ChIP revealed specific DNA:protein interactions for the MolHaps with Sp1 and NF-1, and identified Egr1 interacting exclusively with the -159T allele. We propose new structural features within the OPG promoter region and identified MolHaps being differentially transcriptionally active, and interacting with a proximal polymorphic site.

Poster Session

TNFA-M-RAS-LFA-1 PATHWAY ANALYSIS IN GENOME-WIDE SNP DATA ON PATIENTS WITH MYOCARDIAL INFARCTION

A. Wagner[1], **Jennifer Freyer**[2], A. Medack[2], C. Willenborg[3], C. Loley[3], J. Nahrstedt[3], M. Preuss[3], I.R. König[3], H. Schunkert[2], J. Erdmann[2]

[1]Karolinska Institut, University Stockholm, Stockholm, Sweden, [2]Medizinische Klinik II, [3]Institut für Medizinische Biometry und Statistik, Universität zu Lübeck, Lübeck, Germany

Background: Genome-wide association studies typically focus on single-locus analysis. By contrast, systematic analysis of an affected pathway may be more informative. Recently, we identified the *MRAS* gene as a new risk gene for CAD/MI (rs9818870 P=7.44 x 10^{-13} (OR=1.15; 95% CI=1.11-1.19) by GWAS. The underlying biology is unclear.

We aimed to perform a *MRAS* gene based pathway analysis using Affymetrix genotype data from the German MI Family Studies (GerMIFS I, GerMIFS II, and GERMIFS III (KORA)) comprising a total of 2,000 MI cases and 3,000 population-based controls.

Methods: Identification of relevant genes was undertaken by bioinformatical means of medical literature and internet databases. Available SNPs were investigated in silico in three independent genome-wide SNP data sets genotyped on Affymetrix platforms; missing genotypes were subsequently imputed (GerMIFS I [875 MI cases, 1644 controls] and GerMIFS II [1221 MI cases, 1298 controls].

Results: Thirteen genes were identified in TNFα-M-Ras-LFA-1 pathway. Among them, ten genes carried several SNPs significantly associated with CAD/MI in both GWAS. In *ICAM-1* 17/70 SNPs, in *ITGAL* 8/47 SNPs, in *ITGB2* 25/147 SNPs, in *MRAS* 43/ 91 SNPs , in *Rap1A* 29/276 SNPs, in *RAPL* 30/194 SNPs, in *RIAM* 16/228 SNPs, *TNFα* 13/140 SNPs, in *TNFRSF1A* 7/56 SNPs and in *TNFRSF1B* 14/91 SNPs were significant for CAD/MI [combined p-values ranging from 0.001 - $5x10^{-5}$).

Conclusions: Several SNPs in genes of the TNFα-M-Ras-LFA-1 pathway revealed significant results for CAD/MI in two GWAS. Replication steps are needed to further establish the role of this pathway in the etiology of CAD/MI.

Poster Session

MOLECULAR GENETIC ANALYSIS OF A HUMAN IGF1 PROMOTER P1 VARIATION

Ralph Telgmann[1], C. Dördelmann[2], E. Brand[3], V. Nicaud[4], H. Pavenstädt[3], F. Cambien[4], L. Tiret[4], M. Paul[5], S.-M. Brand-Herrmann[1]

[1]Molecular Genetics of Cardiovascular Disease, Leibniz-Institute for Arteriosclerosis Research, Münster, [2]Institute for Analytical Sciences, Dortmund, [3]University Clinic Münster, Münster, Germany, [4]INSERM UMRS 937, Paris, France, [5]Maastricht University, Maastricht, The Netherlands

IGF1 exerts important endocrine and paracrine functions in the cardiovascular system. We identified the common variant C-1411T in the IGF1 upstream promoter P1, located within several overlapping transcription factor binding sites (TFBS). Using transient transfection assays, we identified this site as a functional enhancer of several, partly overlapping conensus binding sites. The T allele-carrying enhancer, compared to the C allelic portion, exerts significantly reduced or even abrogated activity in SaOs-2 and HepG2, respectively (P-values < 0.0001), as well as in differentiated THP-1 macrophages. EMSA and subsequent Supershift experiments in HepG2 identified c-Jun as binding partner exclusively to the T allele, whereas C/EBPdelta and ICSBP/IRF8 interacted only with the C allelic promoter portion. Furthermore, genotyping of a case-control study for essential hypertension (n=745 hypertensives; n=769 normotensives) for this variant revealed an odds ratio for hypertension of 0.73 ([95%CI 0.58-0.91], P=0.006) associated with the T allele, and normotensives carrying the protective T allele displayed a significant decrease in diastolic (P=0.036) and systolic (P=0.024) blood pressure level. We here report detection of a functional enhancer module in the upstream IGF1 promoter region, which might play a key role in local IGF1 bioavailability. Whether C-1411T is also associated with other IGF1-related disease phenotypes should be evaluated further population studies.

Poster Session

FUNCTIONAL ANALYSES OF THE HUMAN LEUKOTRIENE C_4 SYNTHASE GENE PROMOTER MOLECULAR HAPLOTYPES

Friederike Bruns[1], R.-G. Telgmann[1], B. Schröer[1], B. Schmitz[2], F. Cambien[3], P. Amarenco[4], E. Brand[2], S.-M. Brand-Herrmann[1]

[1]Leibniz-Institut für Arteriosklerosеforschung, [2]Universitätsklinikum Münster, Münster, Germany, [3]INSERM UMRS 937, [4]INSERM U476UMRS 698, Paris, France

LTC_4 Synthase (*LTC_4S*) SNPs have been validated to be associated with cardiovascular disease (CVD), especially stroke phenotypes. *LTC_4S* is a strong mediator of inflammation and affect vascular permeability, its expression being strictly limited to hematopoietic cells. We aimed at analysing the molecular basis of *LTC_4S* promoter control and identifying functional genetic variants with molecularly proven consequences for *LTC_4S* expression.

LTC_4S was genetically scanned in 120 chromosomes of high-risk CVD patients (MolProMD Study). Molecular haplotypes (MolHaps) were identified by individual DNA subcloning. Promoter deletion constructs were generated and transfected in THP1 and U937 cells, the different allelic constellations were introduced by site-directed mutagenesis.

We identified four SNPs (-1783G>A, -1412C>T, -1008G>A, -380A>C) in the 5'-regulatory region, generating six common MolHaps. We found that 2010 bp of the LTC_4s 5´flanking region were transcriptionally active in monocytic cell lines, suggesting a *cis*-active regulatory element located between positions -2010 and -1782 from the TIS. In U937 cells, a second region between positions -1782 and -1560 displayed high transcriptional activity. Transfection analyses of promoter fragments harbouring different MolHaps indicated that MolHaps2 ($P< 0,01$; harbouring the functional SNP -380A>C) and 5 ($P< 0,01$; harbouring the *cis*-located -1783G>A) were significantly less transcriptionally active in THP1 and U937 cells compared to wild type MolHap1.

We identified ~2010 bp of the LTC_4S 5´flanking region to be sufficient for meaningful transcriptional activity in monocytic cell lines, mapped *cis*-active regulatory elements and provide a basis for the molecular functional role of -380A>C in CVD phenotype association.

Poster Session

CORONARY ARTERY CALCIUM SCORE IS A MORE SENSITIVE INDICATOR OF SUBCLINICAL ATHEROSCLEROSIS THAN ANKLE-BRACHIAL INDEX

Andrejs Kalvelis[1], I. Stukena[1], G. Bahs[1], L. Zvaigzne[2], A. Lejnieks[1]

[1]Riga Stradins University, [2]Medical Company 'ARS', Riga, Latvia

Aim: To assess the amount of calcium in coronary arteries (CACscore) by using CT scan and ankle-brachial index (ABI), and their correlations with risk factors of cardiovascular disease (CVD) in patients with adiposity.

Patients and methods: In 200 patients (mean age 52.3±11.7(±SD)) CACscore by CT scan and ABI were measured. The correlations of these parameters with waist circumference (WC), body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP), total cholesterol (TC), low-density and high-density lipoprotein cholesterol (LDL-C and HDL-C), triglycerides (TG), glucose, C-reactive protein (CRP), adiponectin, and leptin were analyzed in accordance with the Pearson correlation coefficient.

Results: In the whole group mean ABI was 0.99±0.14 and CACscore 120±403 without substantial difference between men and women. In persons with adiposity, elevated CACscore was detected, while ABI did not show difference between persons with or without signs of adiposity.ABI and CAC score in different patient subgroups are shown in table. Only in patients with adiposity CACscore had significant correlation with glucose, TC, LDL-C, HDL-C, and adiponectin. Only in patients with increased WC, ABI correlated with SBP and TC while in patients with elevated BMI, ABI did not correlated with any of analyzed parameters.

Conclusions: Elevated CACscore is a more sensitive indicator of CVD risk which correlates with changes of levels of glucose, lipids, and adiponectin. This association is more marked in patients with adiposity.

	Normal BMI	Elevated BMI	p	Normal WC	Increased WC	p	Whole group
ABI	1.01(0.93, 1.07)	1.00(0.94, 1.06)	0.457	1.02(0.94, 1.09)	1.00(0.94, 1.06)	0.210	1.00(0.93, 1.06)
CAC score	0 (0, 31)	7 (0, 100)	0.007	0 (0, 1)	2 (0, 61)	0.013	0 (0, 33)

[ABI and CAC score in different patient subgroups]

Poster Session

LEPTIN TO ADIPONECTIN RATIO IS MODIFIED BY ADIPONECTIN RECEPTOR GENES POLYMORPHISM AND DIET

I. Wybranska[1], M. Malczewska-Malec[1], I. Leszczynska-Golabek[1], B. Kiec-Wilk[1], M. Kwasniak[1], C. Defort[2], J. McMonagle[3], H. Roche[3], **Aldona Dembinska-Kiec**[1]

[1]Department of Clinical Biochemistry, Jagiellonian University Collegium Medicum, Cracow, Poland, [2]INSERM U476, Marseille, France, [3]University College Dublin, Dublin, Ireland

Background: The leptin to adiponectin ratio has been suggested to be predictor of development of metabolic syndrome (MetS) complications. The present study was undertaken to investigate whether the dietary intervention can predict the risk of traits characterizing MetS and affect the impact of leptin/adiponectin ratio in the adiponectin receptor 1 (ADR1) gene variants (rs2275737; rs 10920533) and adiponectin receptor 2 gene variant (rs 1058322) (ADR2) carriers.

Material and methods: The present study was the part of EU LIPGENE human dietary intervention project in patients with MetS.

The 486 MetS subjects were randomly assigned to one of four isoenergetic diets: ; high-fat (38% energy) high-SFA (Diet A); high-fat (38% energy), high-MUFA (Diet B) and two isoenergetic low-fat (28% energy) diets, supplemented with long chain n-3 PUFA (Diet D) or it placebo (Diet C) for 12 weeks.

Results: However before dietary intervention there was any statistical differences in the leptin/adiponectin ratio value between studied genotypic subgroups, the moderate (about 10%) decrease of leptin/adiponectin ratio after 12 weeks of low-fat diet (D and C) was observed in common genotype carriers of ADR1 and ADR2. The significant increase in leptin/adiponectin ratio in the rare AA genotype (rs 109205330) 17,75 vs 9,34 (GG) and rare AA genotype (rs 2275737) 16,01 vs 9,73(CC) was observed.

Conclusion: Low-fat, high carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), enriched in n-3 PUFA is beneficial for MetS , but not with the AA genotype carriers of adiponectin rs109205330 and rs2275737.

Supported by the EU FOOD-CT-2003-505944 LIPGENE and K/ZDS/000620 projects

Poster Session

TIME-SERIES AND GENE CO-EXPRESSION ANALYSES OF TRANSCRIPTIONAL CHANGES IN HUMAN MONOCYTES AFTER ACUTE MYOCARDIAL INFARCTION: THE GERMAN MI FAMILY STUDY

Patrick Linsel-Nitschke[1], H. Sager[1], S. Maouche[2], J. Brocheton[2], J. Erdmann[1], Z. Aherrarhou[1], F. Cambien[2], H. Schunkert[1], Cardiogenics

[1]Medizinische Klinik II, University of Luebeck, Luebeck, Germany, [2]INSERM UMRS 937, Université Pierre et Marie Curie, Paris, France

Background: Myocardial infarction (MI) is associated with an inflammatory reaction and the peripheral recruitment of monocytes to the injured area. We hypothesize that temporal changes in gene expression of circulating monocytes in acute MI will identify genes and pathways of importance in the pathophysiology and clinical course of acute MI.

Methods: Monocyte-RNA from 28 MI patients was obtained at three time points:

1. within 6 hours of onset of chest pain,
2. three days, and
3. 12 weeks after acute MI.

RNA samples were analyzed using Illumina Bead Chip gene expression arrays containing 24,526 probes corresponding to 18,391 genes. To identify patterns of genes modulated between the three time points, different statistical and bioinformatical approaches were used, including mixed linear models and gene set enrichment analysis to elucidate pathways differing significantly between the three time points.

Results: The most significant pathways with multiple genes differentially regulated were:

1. Inhibition of VEGF-mediated angiogenesis through upregulation of Thrombospondin 1.
2. Induction of monocyte adhesion proteins mediated by small GTPases (Rho, Rac, Cdc42).
3. Leukocyte transedothelial migration mediated by chemokines.

Gene set enrichment analysis identified a significant over- representation of genes involved in regulation of myocardial contractility as well as genes involved in cytokine and chemokine mediated signaling.

Conclusion: Expression gene profiling of circulating monocytes in various stages of acute MI enables the identification of genes and pathways contributing to the pathophysiology of MI. This research could lead to the identification of novel therapeutic targets or biomarkers of myocardial ischemia.

Poster Session

ATHEROGENIC DYSLIPIDEMIA IN ADULT CHILDREN OF PATIENTS WITH PREMATURE CORONARY HEART DISEASE. RELATION TO OWN AND PARENTAL RISK FACTORS

Michail Konnov, L. Dobordzhginidze, A. Deev, N. Gratsiansky

Laboratory of Clinical Cardiology, Institute of Physico-Chemical Medicine, Moscow, Russia

Purpose: To elucidate associations between atherogenic dyslipidemia (aDLP) in adult children of patients with premature (onset < 55, men; < 60 years, women) coronary heart disease (PCHD) and their own and parental characteristics.

Methods: We examined members of 182 families - 173 parents-probands (men 65.1%) aged 40-63 years, their 114 consorts (men 23.9%) aged 37-67 years, 218 their children (men 51.4%) aged 18-37 years. Characteristics comprised alcohol, smoking, contraceptives, height, BMI, waist circumference (WC), heart rate, systolic/diastolic BP, total, HDL, LDL cholesterol (C), triglycerides (TG), serum glucose, impaired glucose tolerance, diabetes, prehypertension (PreHT) and hypertension (HT [JNC-7 criteria]), metabolic syndrome (IDF criteria), TG-, LDLC-lowering drugs, and (in parents) education, menstruation status. TG ≥1.7 were defined high, HDLC < 1.03 (men), < 1.29 mmol/l (women) - low. aDLP was defined as high TG + low HDLC. PreHT, HT were combined. Predictors were selected by sex, age adjusted logistic regression.

Results: Characteristics related to aDLP (found in 17/218, children, 7.8%) with p< 0.1 in univariate analysis (WC, BMI, glucose of children; alcohol of proband; TG, HDLC of non-proband) were included into stepwise regression procedure. Independently associated with aDLP were WC (OR top vs 2 bottom tertiles [≥83.0 vs < 83.0 cm] 6.70 [95%CI 1.68-26.7, p=0.007]), glucose of children (OR≥5.0 vs < 5.0 mmol/l 2.90 [95%CI 1.41-5.97; p=0.0038]), alcohol of proband (OR 2.81 [95%CI 1.10-7.23; p=0.032]).

Conclusion: In this group of children with parental PCHD aDLP was independently related to other own metabolic components (WC, glucose) and alcohol consumption of proband.

Poster Session

ADIPOKINES AND SEX HORMONES ARE DIFFERENTLY ASSOCIATED TO CARDIOMETABOLIC RISK FACTORS IN A RANDOMIZED SAMPLE OF ADULT-ELDERLY HEALTHY SUBJECTS

Arrigo Cicero, Brisighella Heart Study Group

Internal Medicine, Aging and Kidney Disease Dept., University of Bologna, Bologna, Italy

Background: Recent evidences show that adipokines could be involved in the development of metabolic syndrome and obesity, being modulated by sexual hormones. Our aim was to evaluate the relationship between some main cardiovascular risk factors and metabolic syndrome (MS) components, adipokines and sexual hormones in a randomized sample of adult-elderly healthy subject.

Methods: We selected a sample of 199 pharmacologically untreated subjects (M: 89; F: 110), aged 62.5±12.4, and representative of their age and class from the Brisighella Heart Study historical cohort. We dosed a range of adipokines and sexual hormones on all subjects and related them to the cardiometabolic parameters of the same subjects.

Results: Among hormones, estrone levels were inversely associated to MS prevalence in women and to blood pressure level in men. Adiponectin level was inversely associated to MS prevalence in both sexes, even if to lower level of different MS components in men and in women. Fasting plasma glucose was inversely associated to adiponectin only in men, while in women it was not associated nor to sexual hormones nor to adipokines. HDL-C was inversely associated to adiponectin and directly to estrone in men, but not in women. Ghrelin was not related nor associated to any MS components in both sexes. Dehydroepiandrosteron was inversely associated to MS prevalence only in women, while testosterone was directly associated to blood pressure only in women.

Conclusion: Sexual hormones and adipokines differently affect the level of different MS component in adult-elderly pharmacologically untreated men and women.

Poster Session

RISK FACTORS ASSOCIATED TO SUBCLINICAL ATHEROSCLEROSIS IN ADOLESCENTS

Egle Silva, L. Chacin, J. Villasmil, G. Ferrer, A. Gonzalez, M. Bracho, C. Esis

Intituto de Enfermedades Cardiovasculares, Facultad de Medicina, Maracaibo, Venezuela

Objective: To estimate the risk factors associated to subclinical atherosclerosis in adolescents.

Methods: We included 163 adolescents, males (n= 71) and females (n= 92), age-mean = 14 years. Age, gender, weight, height, body mass index, waist circumference (WC) and blood pressure (BP) were recorded. It was determined serum glucose, cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Subclinical atherosclerosis (SA) was assessed by carotid intima-media thickness (c-IMT) on B-mode ultrasonography, adolescents with c-IMT abnormal for their age and gender were considered with SA. Correlation analysis was used to detect the association between SA and age, anthropometric data and BP. Logistic regression was applied to determine the risk factors for SA.

Results: The SA prevalence was 30.1% (n=49) in all subjects, and it was 39.4% (n=28) for males and 22.8% (n=21) for females ($p< 0.01$). The correlation analysis evidenced statistically significant associations among SA with systolic BP ($r=0.17$, $p< 0.02$), weight ($r=0.22$, $p< 0.004$), height ($r=0.18$, $p< 0.01$), WC ($r=0.24$, $p< 0.002$), triglycerides ($r=0.18$, $p< 0.01$) and HDL-cholesterol ($r= -0.17$, $p< 0.03$). The factors included for the logistic regression were gender and those found significantly associated in the correlation analysis. The logistic regression analysis showed only WC as statistically significant risk factor for SA [$p< 0.002$ (OR=1.047; Intervals Confidence 95%: 1.017 - 1.077)].

Conclusions: This study evidence high prevalence of SA in adolescents. The measurement of WC, a clinical data easily obtained, is the main risk factor for SA in adolescents. We suggest that WC has the highest atherogenic potential in adolescents.

Poster Session

FIRST APPLICATION OF RISK ADVANCEMENT PERIODS TO LIPID RATIOS

Karl J. Krobot[1], U. Siebert[1,2,3]

[1]Department of Public Health, Information Systems and Health Technology Assessment, UMIT - University of Health Sciences, Medical Informatics and Technology, Hall i. T., Austria, [2]Cardiovascular Research Program, Institute for Technology Assessment and Department of Radiology, Massachusetts General Hospital, Harvard Medical School, [3]Center for Health Decision Science, Department of Health Policy and Management, Harvard School of Public Health, Boston, MA, USA

Purpose: Log-transformed lipid ratios such as the ratio of total cholesterol to HDL cholesterol (log(TC/HDL)) are powerful but unintuitive predictors of cardiovascular risk. We therefore transformed differences in these log ratios into risk advancement periods (RAP; Brenner et al., 1993) that could be used by a physician to explain, for example, to a 50-year-old male that he faces the same risk of suffering a heart attack as does a 65-year-old male with a normal lipid ratio.

Methods: Taking Framingham data as an example for predicting major recurrent coronary events (D'Agostino et al., 2000), the RAP of a TC level elevated by a factor of x in conjunction with an HDL level decreased by a factor of y is calculated as ß(log(TC/HDL))/ß(age)*log(x/y), where ß(log(TC/HDL)) is 0.6738 (men) and 0.8340 (women), and ß(age) is 0.0145 (men) and 0.0225 (women).

Results: We demonstrate that, for example, the RAP of a 1.15-fold TC in conjunction with an 0.8-fold HDL is 16.9 years for a man and corresponds to the addition of the individual RAPs associated with a 1.15-fold TC (6.5 years) and a 0.8-fold HDL (10.4 years), respectively. The risk of major recurrent coronary events for such a person is thus expected to be advanced by an estimated total of 6.5+10.4=16.9 years.

Conclusions: RAPs derived from logarithmized ratios have the specific merit that the RAPs of the individual lipid ratios are additive. Risks associated with combined dyslipidemias can be conveyed more clearly to patients, physicians, payers, and policymakers using RAPs.

Poster Session

N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND METABOLIC SYNDROME

Jou-Wei Lin, F.-C. Chiu, J.-J. Hwang

Cardiovascular Center, National Taiwan University Hospital Yun-Lin Branch, Dou-Liou, Taiwan R.O.C.

Objective: To demonstrate the role of individual components in metabolic syndrome (MetSyn) in associating with N-terminal pro-brain natriuretic peptide (NTproBNP).

Methods: An on-site screening program for MetSyn was held on November 22, 2008 in Yun-Lin, Taiwan. Demographic data, anthropometric measurements, and a series of biochemical tests [glucose, insulin level, lipid profiles, and high-sensitivity C-reactive protein (hsCRP), and NTproBNP] were obtained.

Results: 261 subjects with MetSyn and the other 261 subjects (matched on age, sex, and renal function) without MetSyn aged ≧ 30 were included. The subjects with MetSyn had higher levels of uric acid (mean difference: 0.63 mg/dl, $p < 0.001$), log transformation of hsCPR [mean difference: 0.235 (1.72 fold), $p < 0.001$], but not log(NTproBNP) [mean difference: -0.061 (0.86 fold), $p = 0.153$]. The regression model showed that log(NTproBNP) was positively associated with systolic blood pressure [b= 0.002 per 1 mmHg (1.005 fold), $p = 0.006$], negatively associated with body mass index [b = -0.20 per 1 kg/m^2 (0.96 fold), $p < 0.001$] and triglyceride [b = -0.001 per mg/dl (0.99 fold), p= 0.005], and neutral to waist circumference (p=0.929), fasting glucose (p = 0.916), high-density lipoprotein cholesterol (p = 0.128), and diastolic blood pressure (p = 0.353). Insulin level, when added into this model, was also negatively associated with NTproBNP (p = 0.005).

Conclusion: The results of this study showed that MetSyn was not associated with serum NTproBNP level, due to contradictory effects of each component.

Poster Session

CYANIDIN-3-*O*-B-GLUCOSIDE AND PROTOCATECHUIC ACID STIMULATE GLUT4-MEDIATED GLUCOSE UPTAKE BY UP-REGULATING PPARΓ ACTIVITY IN HUMAN OMENTAL ADIPOCYTES

Roberta Masella[1], B. Scazzocchio[1], A. Pucciarmati[1], C. Filesi[1], R. Vari[1], M. D'Archivio[1], C. Santangelo[1], A. Iacovelli[2], F. Galvano[3], C. Giovannini[1]

[1]Veterinary Public Health and Food Safety, Italian National Institut of Health, [2]Fabia Mater Hospital, Rome, [3]Biochemistry, University of Catania, Catania, Italy

Introduction: A reduced adipocyte response to insulin stimulation is a central event in the establishment of insulin resistance associated with type 2 diabetes, obesity and metabolic syndrome, pathologies that confer an increased risk of cardiovascular disease. The dietary polyphenols cyanidin-3-glucoside (C3G) and its metabolite protocatechuic acid (PCA) have been demonstrated to counteract the development of insulin resistance in mice.

Objective: This study was aimed at unravelling the molecular mechanisms underlying the effects of C3G and PCA on glucose uptake in primary adipocytes isolated from human omental adipose tissue.

Methods: Incorporation of 2-deoxy-D-[^{3}H] glucose in adipocytes incubated for 24h with different concentrations (10-50 µM) of C3G or PCA, was compared to that obtained in cells treated with insulin (0.1µM), and in untreated control cells. GLUT4 expression and membrane translocation, and PPARγ and adiponectin expressions were determined.

Results: C3G and PCA induced insulin-like effects increasing glucose uptake($p < 0.05$) and stimulating GLUT4 translocation ($p < 0.01$) with respect to the controls. Since PPARγ activation has been associated with increased insulin-mediated GLUT4 translocation, we evaluated the nuclear content and activity of PPARγ, as well as the expression of its target gene adiponectin in polyphenol-treated cells. Both polyphenols stimulated a significant increase of PPARγ, (nuclear expression and activity) ($p < 0.05$), and adiponectin (protein expression) ($p < 0.01$), compared to untreated cells.

Conclusion: These findings suggest that C3G and PCA might exert insulin-mimetic effects in human omental adipocytes providing new evidence on the possible role of polyphenols in hindering the development of insulin-resistance and type 2 diabetes.

Poster Session

THE KYNURENINES ARE ASSOCIATED WITH OXIDATIVE STRESS, INFLAMMATION AND THE PREVALENCE OF CARDIOVASCULAR DISEASE IN PATIENTS WITH END-STAGE RENAL DISEASE

Dariusz Pawlak[1], T. Domaniewski[1], M. Mysliwiec[2], K. Pawlak[1]

[1]Monitored Pharmacotherapy, [2]Nephrology and Clinical Transplantation, Medical University of Bialystok, Bialystok, Poland

Objective: Increased oxidative stress (SOX), inflammation and prevalence of cardiovascular disease (CVD) have been reported in end-stage renal disease (ESRD), but their associations with kynurenine (KYN) pathway activation remain unknown.

Methods: We determined the plasma concentrations of tryptophan (TRP), KYN, 3-hydroxykynurenine (3-HKYN); three distinct SOX markers: Cu/Zn superoxide dismutase (Cu/Zn SOD), total peroxide and autoantibodies against oxidized LDL (OxLDL-Ab); and high sensitivity C-reactive protein (hs CRP) as a indicator of inflammation in 146 ESRD patients and healthy controls.

Results: Analysis of TRP degradation through the kynurenine pathway demonstrated that in uremia the concentrations of this aminoacid were decreased by 40-60% in comparison with controls. In contrast, the plasma levels of KYN and 3-HKYN in ESRD patients were increased by 32-96% and 184-306%, respectively. These changes were accompanied by significant increase in the KYN/TRP ratios by 140-240%, and 3-HKYN/KYN ratios by 40-154% in uremics compared to controls. ESRD patients showed a significant increase in Cu/Zn SOD, total peroxide and hs CRP, whereas there was no difference in the mean level of OxLDL-Ab between controls and all patients group. KYN and 3-HKYN were positively associated with inflammation and SOX markers in uremics. Logistic regression analysis showed that age, gender, presence of DM (all $p < 0.001$), elevated hs CRP ($p < 0.01$) and 3-HKYN levels ($p < 0.05$) were independently associated with the presence of CVD in this population.

Conclusions: These results suggest a relationship between kynurenine pathway activation and increased SOX, inflammation and CVD prevalence in ESRD patients.

Poster Session

OXIDIZED-LDL/B2-GLYCOPROTEIN I COMPLEXES AND ANTIPHOSPHOLIPID ANTIBODIES ARE ASSOCIATED WITH DISEASE SEVERITY AND RISK FOR ADVERSE OUTCOMES IN ACUTE CORONARY SYNDROME

Luis Lopez[1], T. Greco[2], A.M. Conti-Kelly[2], R. Anthony[3], J. Geske[1], M. Boisen[1], E. Matsuura[4]

[1]Corgenix, Inc, Broomfield, CO, [2]Internal Medicine, [3]Internal Medicine and Cardiology, Saint Mary's Hospital, Waterbury, CT, USA, [4]Cell Chemistry, Okayama University Graduate School of Medicine, Okayama, Japan

Introduction: Oxidized-LDL binds β2-Glycoprotein I forming pro-atherogenic oxLDL/β2GPI complexes. Patients with severe coronary artery disease (CAD) had significantly higher titers of oxLDL/β2GPI and presented more AE than patients with normal coronaries. Antiphospholipid antibodies (aPL) have also been implicated in atherogenesis. We have reported that the presence of aPL in patients with acute coronary syndrome (ACS) predicts adverse outcomes.

Objective: To evaluate the concurrent significance of both oxLDL/β2GPI complexes and aPL (anti-β2GPI or anti-oxLDL/β2GPI) on the severity (graded angiography) of CAD and AE in 339 ACS patients.

Results: The occurrence of AE increased significantly in aPL+ patients with severe disease (grades IV-VI) than aPL- patients (30.6% vs. 9.0%, $p< 0.005$). The RR for AE of all aPL+ versus aPL- patients was 3.66 (95% CI 1.88-7.14, $p< 0.001$). aPL+ patients with oxLDL/β2GPI in the 2 upper quartiles had significantly more AE than aPL- patients in the same quartiles (26.1% vs. 6.0%, $p< 0.05$), with a relative risk (RR) of 3.2 (95% CI 0.95-10.86, $p< 0.05$). RR in aPL+ patients in the 2 upper quartiles was 6.72 (95% CI 2.4-19.0, $p=0.01$) when compared to aPL- patients in the 2 lower quartiles. The RR of aPL+ patients in the upper oxLDL/β2GPI quartile increased to 13.9 (95% CI 1.8-102.4, $p< 0.001$) when compared to aPL- patients in the lowest quartile.

Conclusions: These results indicate that the co-presence of oxLDL/β2GPI complexes with anti-β2GPI or anti-oxLDL/β2GPI is associated with CAD severity and enhances the risk of AE in ACS, supporting the concept that autoimmunity accelerates atherosclerosis.

Poster Session

THE PROTECTIVE ROLE OF SPECIFIC PROTEIN-1 AGAINST BENZO(A)PYRENINDUCED ATHEROGENESIS

Zhongmao Guo, T. Tang, X. Lin, H. Yang

Meharry Medical College, Nashville, TN, USA

We previously reported that overexpression of Cu/Zn-superoxide dismutase (SOD) and/or catalase reduced benzo(a)pyrene (BaP)-induced atherosclerosis in hypercholesterolemia mice, and that up-regulation of aryl hydrocarbon receptor (AhR) expression was a mechanism by which overexpression of Cu/Zn-SOD and/or catalase accelerated BaP detoxification in mouse aorta endothelial cells (MAECs). The objective of this study was to investigate the regulatory role of specificity protein-1 (Sp1) in AhR expression in MAECs that overexpress Cu/Zn-SOD and/or catalase. Our data demonstrated comparable levels of nuclear Sp1 protein in the transgenic and wild-type MAECs; however, binding of Sp1 protein to the AhR promoter region was more than 2-fold higher in MAECs overexpressing Cu/Zn-SOD and/or catalase than in wild-type cells. Inhibition of Sp1 binding to the AhR promoter by mithramycin A reduced AhR expression and eliminated the differences between wild-type MAECs and three lines of transgenic cells. Functional promoter analysis indicated that AhR promoter activity was significantly higher in MAECs overexpressing catalase than in wild-type cells. Mutation of an AhR promoter Sp1-biding site or addition of hydrogen peroxide to the culture medium reduced AhR promoter activity, and decreased the differences between wild-type MAECs and transgenic cells overexpressing catalase. These results suggest that increased Sp1 binding to the AhR promoter region is an underlying mechanism for up-regulation of AhR expression in MAECs that overexpress Cu/Zn-SOD and/or catalase. These findings contribute to the larger goal of understanding the molecular mechanisms underlying the protective role of Cu/Zn-SOD and/or catalase overexpression against BaP-induced atherosclerosis. This study is supported by NIHR01ES014471 and R01HL089382 (Z.G.).

Poster Session

COLLAGENASE MATRIX METALLOPROTEINASE-8 EXPRESSED IN ATHEROSCLEROTIC CAROTID PLAQUES IS ASSOCIATED WITH SYSTEMIC CARDIOVASCULAR OUTCOME

Wouter Peeters[1,2], F. Moll[2], D. de Kleijn[1], J.P. de Vries[3], A. Newby[4], G. Pasterkamp[1]

[1]Experimental Cardiology, Univesity Medical Center Utrecht, [2]Vascular Surgery, University Medical Center Utrecht, Utrecht, [3]Vascular Surgery, St Antonius Hospital Nieuwegein, Nieuwegein, The Netherlands, [4]Bristol Heart Institute, Bristol, UK

Introduction: Atherosclerotic plaque rupture is the major cause of acute cardiovascular events. There is a need for plaque markers that facilitate detection of the vulnerable plaque and to identify the patient at risk for cardiovascular events. Matrix metalloproteinases (MMP) are prevalent in the arterial wall throughout the arterial system and are associated with plaque destabilization. Therefore we hypothesized that local plaque MMP activity is predictive for atherosclerotic cardiovascular events in other vascular territories.

Methods: Atherosclerotic plaques were obtained from 543 patients undergoing carotid endarterectomy (CEA). Plaques were analyzed for the presence of macrophages, lipid-core, smooth-muscle-cells, collagen, calcification and presence of plaque hemorrhage. MMP-2, MMP-8 and MMP-9 activity levels were assessed within the plaque. Patients underwent follow-up during 3-years after CEA. The primary outcome was defined as the composite of vascular death, non-fatal vascular event and surgical or percutaneous vascular intervention.

Results: In contrast with MMP-2 plaque activity, MMP-8 and MMP-9 plaque activities were associated with an unstable carotid plaque composition and clinical presentation at baseline. Increased plaque MMP-8 activity was associated with an increased risk for the occurrence of secondary manifestations of atherosclerotic disease during follow-up (HR = 1.76, 95%CI [1.25-2.48]) (P=0.001), whereas plaque MMP-2 and MMP-9 were not predictive for systemic cardiovascular events.

Conclusion: In contrast with MMP-2, increased carotid plaque MMP-8 and MMP-9 activity is associated with an unstable plaque phenotype, but specifically collagenase MMP-8 activity is associated with the occurrence of systemic cardiovascular events during follow-up and enables risk stratification with respect to specific arterial territories.

Poster Session

ASSOCIATION BETWEEN CHANGE IN HDL AND VASCULAR EVENTS IN STATIN TREATED PATIENTS: REPORT FROM THE UK GENERAL PRACTICE RESEARCH DATABASE

Lori Bash[1], B. Ambegaonkar[2], C. Wenworth[3], C. May[3]

[1]Global Health Outcomes, Merck and Company, Inc., [2]Global Human Health, Merck & Co., Inc, Whitehouse Station, NJ, [3]Analytic Consulting Solutions, Inc, Wakefield, RI, USA

Background: The independent association between changes in HDL-C and the subsequent risk of cardiovascular events has not been well studied. A retrospective cohort analysis was conducted to evaluate the association between changes in HDL-C and cardiovascular (CV) and/or cerebrovascular (CB) events among patients on statin treatment.

Methods: Patient demographics, clinical characteristics, laboratory data, and CV/CB events, were collected from the UK General Practice Research Database. The association between the risk of an incident event among patients experiencing changes in HDL-C was estimated using multivariate Cox proportional hazards models.

Results: Among 17,923 statin-treated patients who were followed for an average of 1.9 years, there were 815 CV, and 220 CB events. The average change in HDL-C experienced was 0.4 mg/dL, ranging from an average decrease of 11 mg/dL among those in the lowest quartile of change to an average increase of nearly 12 mg/dL among those in the highest quartile of change. CV events occurred at an average overall rate of 21 per 1000 person-years and 17 per 1000 person-years among individuals in the highest quartile of change in HDL-C levels. Adjusted Cox regression estimated a 6% decrease in hazards (HR, 0.94; 95% CI, 0.90, 0.98) of a subsequent CV event associated with each 5 mg/dL increase in HDL-C. Similar results were observed when assessing the association with changes in HDL-C and the composite outcome of CV/CB event.

Conclusion: Among statin-treated patients from UK clinical practices, increases in HDL-C were associated with a significantly decreased relative risk of experiencing cardiovascular events.

Poster Session

INTERLEUKIN-6 IS STRONGER ASSOCIATED WITH ALL-CAUSE AND CARDIOVASCULAR MORTALITY THAN C-REACTIVE PROTEIN, SERUM AMYLOID A AND FIBRINOGEN (THE LURIC STUDY)

Hubert Scharnagl[1], T. Stojakovic[1], G. Weihrauch[1], B.R. Winkelmann[2], B.O. Boehm[3], W. Maerz[1,4]

[1]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria, [2]Cardiology Group Frankfurt-Sachsenhausen, Frankfurt/Main, [3]Department of Medicine, Division of Endocrinology, University of Ulm, Ulm, [4]Synlab Center of Laboratory Diagnostics, Heidelberg, Germany

There is abundant evidence that inflammation plays a regulatory role in the developement and destabilisation of atherosclerotic plaques. Interleukin-6 (IL-6) stimulates the production of acute-phase reactants, including C-reactive protein (CRP), serum amyloid A (SAA) and fibrinogen in the liver. The Ludwigshafen Risk and Cardiovascular Health (LURIC) Study is a prospective cohort study of 3247 subjects who underwent coronary angiography. The association of inflammatory markers with mortality was analysed using Cox proportional hazard models. After a median follow-up of 7.75 years, 753 patients died, including 472 from cardiovascular causes. Compared with subjects in the lowest quartile of IL-6, the HRs in the highest quartile were 2.35 (95%CI 1.70-3.25, p< 0.001) for total mortality and 2.09 (95%CI 1.42-3.09, p< 0.001) for cardiovascular mortality in analysis adjusted for cardiovascular risk factors. An increase of 1SD in IL-6 concentration was associated with adjusted HR for total mortality of 1.36 (95% CI 1.25-1.48, p< 0.001). Comparable associations were observed for CRP (HR 1.27, 95%CI 1.17-1.38, p< 0.001), SAA (HR 1.19, 95%CI 1.07-1.33, p< 0.001), and fibrinogen (HR 1.13, 95%CI 1.03-1.14, p=0.006). IL-6 retained its prognostic importance after adjustment for each of the other inflammatory markers. In contrast, CRP, SAA, and fibrinogen were not significantly associated with total mortality after adjustment for IL-6. IL-6 levels were significantly associated with all-cause and cardiovascular mortality in patients undergoing coronary angiography. Our results suggest that IL-6 is a stronger predictor of total and cardiovascular mortality than CRP and that its prognostic value is independent of other markers of inflammation.

Poster Session

LIPOPROTEIN (A) AND THE RISK OF CARDIOVASCULAR DISEASE - SYSTEMATIC REVIEW AND META ANALYSIS OF PROSPECTIVE STUDIES INCLUDING 161222 INDIVIDUALS

Bernd Genser[1], K. Dias[1], R. Siekmeier[2], T. Stojakovic[3], T. Grammer[4], W. März[4]

[1]BGStats Consulting, Wagersbach - Graz, Austria, [2]Abteilung Medizinprodukte, Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn, Germany, [3]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, Graz, Austria, [4]Synlab Center of Laboratory Diagnostics, Eppelheim, Germany

Background: Several studies have investigated the role of Lipoprotein(a) [Lp(a)] as a risk factor for cardiovascular disease and have produced controversial results.

Methods: We conducted a literature review in the database MEDLINE retrieving prospective studies (cohort studies and nested case control studies) with at least one year of follow-up that were published until September 2007 and investigated the prognostic value of Lp(a) concentrations on cardiovascular risk and mortality. From each study association measures (relative risks or odds ratios) were extracted and standardised to compare the top third with the bottom third of the study specific Lp (a) distribution. Pooled summary estimates were calculated by using random effects meta analysis techniques.

Results: We identified 63 prospective studies involving a total of 161222 patients. For the endpoint combined coronary events (n=33 studies) we found a combined risk ratio (RR) of 1.61 (95% CI: 1.41 to 1.84), with similar findings when the analyses were restricted to 13 population based studies (RR=1.49, 95% CI: 1.22 to 1.82). The effect of elevated Lp(a) on coronary risk was even higher when previous CAD was present (n=6 studies, RR=2.34 (1.41-3.97)). Further, we found a significant effect on mortality in population based studies (n=3 studies, RR=1.44 (1.02-2.05)). The effect on the endpoint stroke was not significant on the 5% level (n=15 studies, RR=1.13 (0.98-1.30)).

Conclusion: This meta analysis of prospective studies demonstrates a clear association between elevated Lp(a) levels and coronary risk. Further, Lp (a) affected total mortality in population based studies.

Poster Session

FASTING SERUM APOLIPOPROTEIN B/A-I RATIO PREFERENTIALLY PREDICTS MAJOR ADVERSE CARDIOVASCULAR EVENTS IN THE GENERAL POPULATION INDEPENDENTLY OF ALBUMINURIA AND CRP

Paul J.W.H. Kappelle[1], R.T. Gansevoort[2], B.H.R. Wolffenbuttel[1], R.P.F. Dullaart[1], PREVEND

[1]Endocrinology, [2]Nephrology, University Medical Center Groningen, Groningen, The Netherlands

Background: Apolipoproteins (apos) have been suggested as alternative measures to lipoprotein cholesterol levels in cardiovascular risk prediction. We compared the strength of relationships of major adverse cardiovascular events (MACE) with fasting lipoproteins, apos and their ratios in the general population. The extent to which these relationships were modified by traditional risk factors, high sensitivity C-reactive protein (CRP) and albuminuria was also studied.

Subjects and methods: A prospective case-cohort study was performed among 6948 subjects (PREVEND cohort) without previous cardiovascular disease and who did not use lipid lowering drugs initially. Fasting serum total cholesterol, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, apoB, apoA-I, apoA-II and triglycerides were measured at baseline. The composite endpoint was incident MACE.

Results: 362 cardiovascular events occurred during a median follow-up of 7.85 years. MACE was associated with all pro- and anti-atherogenic lipoprotein measures, apos and their ratios in age-and sex-adjusted Cox proportional hazard analyses (P=0.018 to P< 0.001); these relationships were similar after triglyceride adjustment. Pair-wise comparison between single (apo)lipoprotein measures and ratios revealed that the hazard associated with the apoB/apoA-I ratio was higher compared to other pro-atherogenic measures, including total cholesterol/HDL cholesterol. The risk associated with the apoB/apoA-I ratio was only modestly attenuated by traditional risk factors (hypertension, diabetes, obesity, smoking), CRP and albuminuria (fully adjusted Hazard Ratio:1.24; 95 % CI, 1.12-1.38, P< 0.001); no interactions of the apoB/apoA-I ratio with albuminuria and CRP on risk were observed.

Conclusion: The fasting serum apoB/apoA-I ratio preferentially predicts MACE in the general population, independently of albuminuria and CRP.

Poster Session

MARKERS OF CHOLESTEROL METABOLISM ARE ASSOCIATED WITH CAROTID INTIMA-MEDIA THICKNESS

Oliver Weingärtner[1], D. Lütjohann[2], K. Rogacev[3], L. Blömer[3], Y. Grenner[4], S. Gräber[5], C. Ulrich[3], M. Girndt[3], M. Böhm[1], D. Fliser[3], U. Laufs[1], G. Heine[3]

[1]Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, [2]Institut für Klinische Chemie und Pharmakologie, Universitätsklinikum Bonn, [3]Klinik für Innere Medizin IV, [4]Klinik für Innere Medizin II, [5]Institut für Medizinische Biometrie, Epidemiologie und Informatik, Universitätsklinikum des Saarlandes, Homburg, Germany

Objective: Total cholesterol is related to atherosclerosis, but it is unclear whether differences in cholesterol absorption and synthesis play a role. In this study markers of cholesterol homeostasis were studied in relation to intima-media thickness (IMT) of the carotid arteries.

Methods: Carotid IMT was measured by duplex ultrasound in 583 hospital employees aged 25-60 years without known cardiovascular disease, diabetes mellitus and lipid-modifying medications. The serum concentrations of plant sterols, as indicators of cholesterol absorption and lathosterol, indicating cholesterol synthesis as well as cholesterol were determined by gas-liquid chromatography.

Results: IMT correlated with cholesterol concentrations (r=0.22, P< 0.0005) and lathosterol concentrations (r=0.25, P< 0.0005), but showed no correlation to plant sterol levels. Stratifying subjects by sterol levels, we found that IMT measurements increased continuously over quintiles of cholesterol (P for trend; p< 0.0005) with subjects in quintiles 5 and 4 having higher IMT measurements compared to those in quintile 1 (0.46+/-0.09 mm and 0.44+/-0.09 mm vs. 0.41+/-0.06 mm, P< 0.005 and P< 0.0005, respectively). Furthermore, IMT measurements increased continuously over quintiles of lathosterol (P for trend; p< 0.0005) with significantly higher values in quintiles 5 and 4 compared to quintile 1 (0.45+/-0.08 mm and 0.44+/-0.07 mm vs. 0.41+/-0.06 mm, P< 0.005 and P< 0.0005), but showed no difference over quintiles of plant sterol levels.

Conclusions: In this middle-aged, statin-naïve population, increased endogenous cholesterol synthesis but not cholesterol absorption positively correlated with early atherosclerosis as judged by carotid IMT measurements.

Poster Session

THE ASSOCIATION OF METABOLIC SYNDROME WITH CORONARY HEART DISEASE AND STROKE

Jiro Suwa

Preventive Medicine, St. Luke's International Hospital, Tokyo, Japan

Objective: The objective of this study was to examine the association of metabolic syndrome (MS) with coronary heart disease (CHD) and stroke.

Methods: This prospective study was comprised of 13,839 individuals (6813 men; mean age 49.2 years, 7026 women; mean age 47.5 years) who underwent annual health checks at our institution in 2002. Individuals with a history of CHD or stroke or who were treated with medications for life style-related disease (list here, example: “diabetes, hyper blood pressure, dyslipidemia”, etc.) were excluded. The prevalence of MS was analyzed using International Diabetes Federation criteria for Japanese (waist circumference ≥90 cm for men, ≥80 cm for women).

Results:

(1) The prevalence of MS in men was 10.6% and 6.0% in women.

(2) During follow-up (mean 4.0±1.4 years), there were 108 new CHD or stroke diagnoses (71 men, 37 women; 58 CHDs, 50 strokes).

(3) Age-adjusted multivariable hazard ratio (HR) for CHD associated with MS was 2.333(95% CI; 1.028-5.290, $p< 0.05$) in men.

HR for stroke associated with MS was 2.457(95% CI; 1.010-5.977, $p< 0.05$) in men. There were no significant HRs for CHD and stroke in women.

Conclusion: Our findings suggest that MS is very likely a major determinant for developing CHD and stroke in men but that this relationship remains unclear in women.

Poster Session

THE P22-PHOX C242T POLYMORPHISM IS ASSOCIATED WITH LOWER LEFT VENTRICULAR MASS IN HYPERTENSIVE PATIENTS

Roberto Schreiber[1], M.C. Ferreira-Sae[1], M.L. Sales[2], J. Cipolli[1], C. Piveta[1], J.R. Matos-Souza[1], K. Franchini[1], W. Nadruz Jr[1]

[1]Department of Internal Medicine, School of Medical Sciences, State University of Campinas., Campinas, [2]Department of Internal Medicine, School of Medical Sciences, Ouro Preto University, Ouro Preto, Brazil

Background and aims: Reactive oxygen species play an important role in the development of cardiovascular disease. Polymorphisms of the p22-phox gene have been associated with cardiovascular disease, but available data are conflicting. Since hypertensive patients with left ventricular (LV) hypertrophy exhibit an unfavorable prognosis and the p22-phox C242T polymorphism has been associated with hypertension, this study investigated the relationship between this variant and hypertensive end-organ damage.

Methods: We evaluated 443 patients (268 women and 175 men) by clinical history, physical examination, anthropometry, analysis of metabolic, inflammatory and echocardiography parameters and p22-phox C242T polymorphism genotyping. Data are presented as the mean ± standard error.

Results: Genotype frequencies in the whole population were consistent with the Hardy- Weinberg equilibrium (TT= 60, CT= 190, CC= 193; x^2 =1.45, p=0.228). Subjects with the CC genotype presented lower posterior wall thickness (10.5±0.1 vs 10.9±0.1mm; p=0.009), LV mass/body surface area (138.2±2.8 vs149.2±2.9g/m^2; p=0.009) and LV Mass/height (156.2±3.4 vs 171.1±3.6g/m; p=0.004) than those exhibiting CT+TT genotypes. These results were confirmed by stepwise regression analyses adjusted for systolic and diastolic blood pressure, age, body mass index, diabetes mellitus, menopause status and use of antihypertensive medications.

Conclusions: The CC genotype of the p22-phox C242T polymorphism is associated with reduced LV mass in hypertensive subjects. These data suggest that genetic variation in NADPH-oxidase components may modulate LV remodeling in hypertensive patients.

Poster Session

POSTPRANDIAL BETA CELL RESPONSIVENESS - DOES IT HAVE A PROTECTIVE ROLE ON THE DEVELOPMENT OF DIABETIC COMPLICATION?

Chul Sik Kim[1], C.W. Ahn[2], M.H. Cho[2], E.J. Lee[2], S.W. Park[3], S.K. Kim[3], S.H. Kim[4], S.J. Han[5], S.H. Lee[6], S.E. Park[7], Y.J. Choi[8], S.H. Ji[2], K.B. Huh[8]

[1]Hallym University Medical College, Anyang, [2]Yonsei University Medical College, Seoul, [3]CHA Medical University, Pocheon, [4]Inha University Medical College, Incheon, [5]Ajou University Medical College, Suwon, [6]Gachon Medical College, Goyang, [7]SungKyunKwan University, [8]Huh's Diabetic Clinic, Seoul, Republic of Korea

Objective: The aim of this study was to evaluate the relationship between insulin secretion both during fasting and 2 hour postprandial and chronic complications in patients with type 2 diabetes mellitus (T2DM).

Patients and methods: We conducted a meal tolerance test in 3944 T2DM patients and calculated fasting and postprandial beta-cell responsiveness. According to their fasting, postprandial and ΔC-peptide levels, the subjects were divided into four groups, and metabolic parameters were analyzed.

Results: In the group with highest fasting C-peptide quartile, their duration of diabetes was shortest. Their BMI, waist circumference, visceral fat thickness, blood pressure, insulin resistance state (assessed by short insulin tolerance test), carotid intima-media thickness (CIMT) were higher than those of other groups. However, frequency of retinopathy, neuropathy, and fasting glucose, postprandial glucose, and A1C were not different among the four groups. In the group with highest ΔC-peptide quartile, subjects' duration of diabetes was shorter, and their fasting glucose, postprandial glucose, and A1C were lower than those of the other three groups. However, blood pressure, CIMT, and insulin resistance state were not different among the four groups. Fasting C-peptide was found to be correlated with insulin resistance (r=-0.302), CIMT (r=0.800). ΔC-peptide was correlated with A1C (r=-0.319), urine albumin excretion (r=0.129).

Conclusion: Our finding indicates a relationship between fasting C-peptide level and carotid atherosclerosis in the patients with T2DM. Moreover, ΔC-peptide was observed to exert greater relationship with glycemic control in T2DM.

Poster Session

MYELOPEROXIDASE IS ASSOCIATED WITH BLOOD PRESSURE ON A BACKGROUND OF HYPERGLYCEMIA OR OXIDATIVE STRESS

Tom Teerlink[1], L.P. van der Zwan[1], J.M. Dekker[2], C.D.A. Stehouwer[3], R.J. Heine[4], P.G. Scheffer[1]

[1]Department of Clinical Chemistry, [2]EMGO Institute, VU University Medical Center, Amsterdam, [3]Department of Internal Medicine, Maastricht University Medical Center, Maastricht, [4]Department of Endocrinology, VU University Medical Center, Amsterdam, The Netherlands

Background: Scavenging of the vasodilator nitric oxide by myeloperoxidase activity in the vasculature may contribute to hypertension. Because hydrogen peroxide is a co-substrate of myeloperoxidase, (hyperglycemia-induced) oxidative stress may strengthen the relation between myeloperoxidase and blood pressure.

Methods: We investigated the relation between myeloperoxidase and blood pressure and its modification by oxidative stress in a population-based cohort of elderly subjects with normal glucose metabolism (n=267), impaired glucose metabolism (n=189), and type 2 diabetes (n=290).

Results: In an age- and sex-adjusted linear regression model, plasma myeloperoxidase was positively associated with systolic blood pressure (2.10 mmHg per one standard deviation increment of myeloperoxidase [95% confidence interval: 0.66 to 3.54]), and this association was stronger at higher levels of fasting glucose (0.61 [-1.70 to 2.93], 1.33 [-1.43 to 4.10], and 3.42 [1.01 to 5.82] for increasing tertiles of glucose) and higher plasma levels of oxidized LDL (0.92 [-1.31 to 3.14], 2.00 [-0.71 to 4.70], and 3.58 [0.98 to 6.19] for increasing tertiles of oxidized LDL). Likewise, the relation between myeloperoxidase and systolic blood pressure was strongest under conditions associated with oxidative stress, like obesity, cardiovascular disease, low HDL-cholesterol and smoking. The strength of these associations was only marginally attenuated by adjustment for other cardiovascular risk factors.

Conclusions: Myeloperoxidase is positively and independently associated with blood pressure and this association is strongest in subjects with (hyperglycemia-induced) oxidative stress. These observations, together with emerging evidence that myeloperoxidase-derived oxidants contribute to initiation and propagation of cardiovascular disease, identify myeloperoxidase as a promising target for drug development.

Poster Session

OPTIMAL TYPE 2 DIABETES MANAGEMENT INCLUDING BENCHMARKING AND STANDARD TREATMENT. BASELINE EUROPEAN RESULTS

Michel Hermans[1], E. Muls[2], G. Michel[3], M. Elisaf[4], C. Brotons[5], A. Matthys[6]

[1]Endocrinology, UCL St Luc, Brussel, [2]Endocrinology, KUL Leuven, Leuven, Belgium, [3]Endocrinology, Luxembourg Hospital, Luxembourg, Luxembourg, [4]Internal Medecine, University of Ioannina, Ioannina, Greece, [5]Catalan Health Department, Sardenya Primary Health Care Centre, Barcelona, Spain, [6]Medical, AstraZeneca, Brussels, Belgium

Background: Diabetes complications have an important impact on survival, quality of life and healthcare costs. Effective treatments and interventions reduce this burden and improve patient's care quality. Benchmarking incorporates two-sided feedback of physician's individual performance graded alongside the current mean achievement of a peer group, as well as patient's target attainment.

Methods: Optimise is a non-interventional study conducted in 6 European countries. The aim was to study the effect of benchmarking on quality of care in type 2 diabetes patients throughout a 12-month period. Parameters of interest were percentage of patients achieving pre-set targets according to European guidelines (2007) for HbA1c (< 7%), LDL-cholesterol(LDL-C< 100 mg/dl) and systolic blood pressure (SBP< 130 mmHg).

Results: 2477 patients were randomized to the benchmarking group and 1503 patients to the no feedback group. Both groups were highly superposable for baseline demographic and diabetes-related parameters. Mean age was 66 years; 55% were males. Mean age at diabetes diagnosis was 57 years, with a mean 8 years since diagnosis. Target HbA1c was reached in 55% of patients. 41% achieved LDL-C target, lipid-lowering drugs (LLD) were given to 67%, with statins most frequently used: simvastatin (40%), rosuvastatin (27%) and atorvastatin (23%).78% were hypertensive (77% treated), with 27% at SBP goal. Only 5 % of patients reached all 3 preset targets.

Conclusions: Quality of care in type 2 diabetes patients at baseline was suboptimal. Interventions to improve levels of care are necessary. OPTIMISE study will evaluate the effect of benchmarking on quality of care in general practice.

Poster Session

LOW BODY MASS INDEX BOOSTS THE NEGATIVE IMPACT OF HYPERGLYCEMIA ON SURVIVAL IN THE ELDERLY

John Ohrvik[1], P. Hedberg[2], G. Nilsson[3]

[1]Medicine, Karolinska Institutet, Stockholm, [2]Physiology, Central Hospital Vasteras, Vasteras, [3]Centre for Clinical Research, Uppsala University, Uppsala, Sweden

Background: An inverse relation between excess weight and all-cause and cardiovascular mortalities has been found in patients with established coronary disease. This observation is referred to as the 'obesity paradox'. Hyperglycemia predisposes to coronary disease. Studies on these relations in the elderly are scanty. We hypothesized that the negative effect of increased fasting plasma glucose (FPG) are magnified in elderly individuals with low BMI.

Methods: We analyzed prospectively associations of BMI, FPG and their interaction with all-cause mortality in a cohort of 222 women and 210 men comprising 70% of a random sample of 75-year-olds from the general population of a Swedish city (median follow-up 10.6 years). Stratified analyses of all-cause mortality for individuals with BMI below and above 25 kg/m^2 were also performed.

Results: FPG (mmol/L; median and interquartile range) was 5.8 (5.4-6.2) and 6.0 (5.5-6.7) in individuals with BMI below (n=192) and above (n=240) 25 kg/m^2. The incidence of all-cause mortality was 43.2 and 40.7 per 1000 person-years. Relative risks (RR) according to Cox regressions, adjusting for sex, smoking, hypertension, and myocardial infarction were:

BMI kg/m^2	**AdjustedRR (95% CI) /mmol/L FPG**
≤25	1.29 (1.11-1.50)
>25	1.05 (0.97-1.14)
20	1.22 (1.04-1.41)
30	0.98 (0.85-1.12)

[Table 1]

Conclusion: In a general population of 75-year-olds, there is a strong positive association between FPG and all-cause mortality in individuals with low or normal BMI which disappears in obese individuals. The present results indicate in light of previous findings that the pathophysiology of hyperglycemia in lean individuals might be of a different more aggressive type.

Poster Session

BODY WEIGHT AND WAIST CIRCUMFERENCE INFLUENCE OVER BLOOD PRESSURE CONTROL IN PRIMARY CARE IN SPAIN. TAPAS STUDY

Teresa Mantilla[1], C. Suarez[2], A. Galgo[3], M. Leal[4], J. Arana[5], M. Hernandez[6], E. Lara[7], M. Marco[8], M.L. Asensio[9], J. San Jose[10]

[1]Primary Care Center Prosperidad, [2]Internal Medicine, Hospital Universitario La Princesa, [3]Primary Care Center Espronceda, [4]AstraZeneca, Madrid, [5]Primary Care Center Segundo Ensanche, Pamplona, [6]Primary Care Center Tabernes Blanques, Valencia, [7]Primary Care Center Sector Sur, Cordoba, [8]Primary Care Center Burjassot, Valencia, [9]Primary Care Center Fuentelarreina, Madrid, [10]Primary Care Center Metges de Capçalera, Sabadell, Spain

Aims: Assess the influence of weight and waist circumference (WC) on blood pressure (BP) control in a cohort of hypertensive patients followed during a year.

Methods: Retrospective study including 1678 hypertensive patients classified in 4 cohorts: cohort 1: BP not controlled in first and last visit: cohort 2: BP not controlled in first but controlled in last visit; cohort 3: BP controlled in first and lost control in the last visit; cohort 4: BP controlled in both visits. Good BP control was < 140/80 mmHg and < 130/80 mmHg in diabetics.

Results: Mean age 64 years, 53% men. In cohort 4, weight was decreased. The mean weight was lower in cohort 4 in first and last visit ($p< 0,05$) and there was a decrease in weight in cohort 2 ($p=0,0467$). Regarding the WC it was also lower in cohort 4 in the first and last visit, being the highest in the cohort with no BP control in both visits ($p=0,0097$). Moreover, cohort 2 was also the one with a greater decrease in WC between the two visits ($p=0,0095$), however WC increases in cohort 3.

Conclusions: Patients that meet the objectives for BP during the study and those who manage to obtain them after a year have reduced weight and WC. In contrast, those who had a good BP control and lose it after a year increased their weight and WC. The decrease in weight and WC affects the achievement of optimal BP control.

Poster Session

PREVALENCE OF FEATURES OF THE METABOLIC SYNDROME IN HEALTHY WHITE-COLLAR EMPLOYEES WITH OR WITHOUT FATTY LIVER DISEASE

Jutta Haas[1], S. Teufel-Sies[2], S. Mack[1], E. Becker[2], B. Knebel[3], J. Kotzka[3], A. Frisch[1], A. Burchard[1], M. Merkel[1], T. Stein[2], D. Müller-Wieland[1]

[1]Asklepios Klinik St. Georg, [2]Diagnostic Center Fleet-Insel, Hamburg, [3]German Diabetes Center, Düsseldorf, Germany

Objective: Non alcoholic fatty liver (NAFL) is the most common chronically liver disease and often associated with features of the metabolic syndrome. There is little knowledge about the prevalence of single components of the metabolic syndrome in healthy individuals with or without fatty liver disease.

Methods: Data of > 1500 healthy, male white-collar employees (30 - 60 year old) were collected.

Results: Sonographic analyses indicated fatty liver in 32%, approximately 50% of these individuals showed triglyceride levels (TG) >150 mg/dl and 12% had HDL-levels below 40 mg/dl. More than 67% of probands showed hypertension with values above 130/85 mmHg and fasting plasma glucose levels exceeding 110 mg/dl were detected in 13% of individuals. These data indicated a more than twofold increased prevalence of dyslipidemia and hypertension in patients with fatty liver disease. Interestingly, pathologically increased fasting blood glucose values were detected in only 2.5% of individuals without fatty liver, but occurred with a 7-fold higher prevalence in patients with fatty liver disease. Focusing on three prominent parameters crucial for the metabolic syndrome, i.e. increased TG levels, hypertension and increased fasting blood glucose >110 mg/dl, the criteria were fulfilled in 14% of individuals with fatty livers, but only in 2% of the non fatty liver group. Reducing the critical value for impaired fasting glucose from >110 mg/dl to >100 mg/dl even doubled the prevalence of this risk factor pattern in the fatty liver group investigated.

Conclusion: Individuals with fatty livers should routinely be screened for criteria of the metabolic syndrome.

Poster Session

LIPID PROFILE IN JAPANESE PATIENTS WHO DEVELOPED CORONARY ARTERY DISEASE DURING STATIN THERAPY

Kei Kojima, A. Motoyama, M. Watanabe, H. Tanabe, H. Aoki, R. Saito, K. Fujibayashi, R. Sato, H. Akao, Y. Kawai, M. Kitayama, K. Kajinami

Department of Cardiology, Kanazawa Medical University, Uchinada, Japan

Background: Statins are widely used for coronary artery disease primary prevention. Treatment guidelines recommend target LDLC/nonHDLC levels graded according to the number of co-existing risk factors. However, current status of lipid profile in the relation with statin therapy remains scarce.

Aim & methods: To investigate this, we surveyed coronary risk profile of patients (n=464, M/F=340/124, mean 69yr) who received coronary intervention for the first time from Jan. 2006 to Dec. 2008, with special reference to preceding statin administration and the number of co-existing coronary risk factors other than LDLC/nonHDLC (0, 1-2, and ≥3).

Results: Among non-statin users (n=275), mean levels of non-HDLC (135, 148, and 162mg/dl) and triglyceride (TG) (94, 109, and 150mg/dl) both increased significantly ($p < 0.01$) by the number of risk factors. In patients receiving mild statins (prava, simva, fluva) (n=52), mean levels of nonHDLC, TG, and HDLC were all below the target levels, and no significant difference was observed across these 3 groups. In patients receiving strong statins (atorva, pitava, rosuva) (n=137), no significant difference was observed across 3 groups regarding mean levels of nonHDLC (139, 154, 151 mg/dl), TG (97, 149, 156 mg/dl), nor HDLC (38, 48, 45 mg/dl).

Conclusion: Lipid profile in patients who developed coronary artery disease during statin therapy was very close to the level recommended in the treatment guideline. More aggressive lipid management and/or more intensive modification of co-existing risk factors would be necessary to improve prevention efficiency.

Poster Session

PREVALENCE OF LOW HDL-C IN STATIN-TREATED PATIENTS WITH CARDIOVASCULAR DISEASE. THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], C. Guijarro[3], E. Alegria[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hopital Clinco Universitario, Santiago de Compostela, Santiago de Compostela (La Coruña), [3]Hospital de Alcorcon, Alcorcon (Madrid), [4]Clinica Universitaria de Pamplona, Pamplona (Navarra), [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Introduction: Secondary prevention attempts to reduce cardiovascular (CV) events; multiple risk factors(hypertension, obesity, diabetes or dyslipidemia)should be controlled.LDL-C is the target to treat dyslipidemia, but HDL-C is also a CV risk factor especially on coronary heart disease (CHD).In this analysis we assess the prevalence of low HDL-C in statin-treated patients with cardiovascular disease (CVD) included in DYSIS study.

Methods: Analysis of 3710 Spanish DYSIS patients (22063 partients in Europe and Canada)≥ 45 year-old treated with statins.We used the ATP-III recommendations to classify patient´s risk and define the LDL-C goal and normality or not of the HDL-C and triglycerides levels.

Results: In 3710 Spanish patients:35.7% had established CVD (62.1% men and 34.0% women; p< 0,001),76.3% were hypertensive, 45.4% diabetics, 17.6% smokers and 24.6% with family history of premature coronary heart disease (CHD).LDL-C was not at goal in 51.1% of CVD patients(vs. 49.9% of non-CVD patients).The prevalence of low HDL-C was 35.7%, it was higher than in patients without CVD (26.4%, p< 0.001) and 36.8% had elevated TG(≥150 mg/dL) compared to 38.2% in patients without CVD.Lipid profiles were abnormal on 10.4% of CVD patients significantly higher in women vs. men (14.3% vs. 8.4%, p=0.01).

Conclusions: In this analysis, almost 50% of patients with CVD do not achieve LDL-C goal, despite statin treatment and a considerable number show an abnormal lipid profile. Low HDL-C could be an important cardiovascular risk factor as the prevalence of HDL-C is higher in patients with cardiovascular disease. Treatment of low HDL-C added to statin therapy may help to prevent the development of cardiovascular events.

Poster Session

RESIDUAL RISK IN SPANISH STATIN TREATED PATIENTS WITH LDL-C AT GOAL. THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], E. Alegria[3], C. Guijarro[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hopital Clinco Universitario, Santiago de Compostela, Santiago de Compostela, [3]Clinica Universitaria de Pamplona, Pamplona, [4]Hospital de Alcorcon, Alcorcon, [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Introduction: Statin reduce cardiovascular risk(CVR) associated with LDL-C, but have limited action on other lipid risk factors(low HDL-C and elevated TG). Some studies suggest that patients with an abnormal lipid profile(high LDL-C, TG and low HDL-C) have higher CVR than those with high levels of LDL-C alone. In this analysis we assess the prevalence of CV disease(CVD) in patients with LDL-C at goal plus other risk factors(RF), regarding HDL-C and TG levels.

Methods: Analysis of 3710 Spanish patients of DYSIS(22063 patients treated with statin in Europe and Canada). We used the ATP-III recommendations to classify patient´s risk and define the LDL-C goal and normal HDL-C and TG levels.

Results: In 3710 patients:69.0% with hypertension, 39.0% diabetics and 51.3% with several RF.LDL-C was not at goal in 51.3% of patients, 29.8% had low HDL-C and 37.6% elevated TG levels.36.8% of patients had several abnormal lipid parameters and only 26.7% had a normal lipid profile.The prevalence of CVD in patients with LDL-C at goal+RF was higher in those that also had low HDL-C and high TG levels than those who had a normal lipid profile(36.7% vs. 19.7% [women], p< 0.001; 50.0% vs. 44.4% [men], p=0.31).

Conclusions: In this analysis LDL-C is not at goal in almost 50% and 30% had low HDL-C.Despite having LDL-C at goal,other lipid parameters that could increase CVR are important.CVD prevalence is higher in patients with low HDL-C and high TG.Therapy involving the whole lipid profile may reduce the persistent risk associated with lipid factors abnormalities that remain after statin treatment.

Poster Session

THE ASSOCIATION OF ALBUMINURIA WITH ANGIOGRAPHICALLY DETERMINED CORONARY ATHEROSCLEROSIS

Philipp Rein[1,2,3], C.H. Saely[1,2,3], A. Vonbank[1,2,3], S. Beer[1,2,3], S. Aczel[1,2], V. Jankovic[1,3], C. Boehnel[1,3], L. Risch[1], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Introduction: Albuminuria is associated with atherothrombotic events and all-cause mortality in patients with and without diabetes. However, it is not known whether albuminuria is associated with atherosclerosis per se in the same manner.

Methods: The population of this study included 914 consecutive patients undergoing coronary angiography for the evaluation of stable CAD. Stenoses with lumen narrowing of ≥50% were considered significant. Urinary albumin to creatinine ratio (ACR) was measured in all patients. Elevated urinary albumin excretion (UAE) was defined as an ACR of 30µg/mg or greater. Microalbuminuria was defined as 30 to 300µg albumin / mg creatinine, macroalbuminuria as an ACR of 300 µg/mg or greater.

Results: The prevalence of stenoses ≥50% was significantly higher in patients with an elevated UAE compared to those without (65.9 vs. 51.4%; $p < 0.001$). In a fully adjusted logistic regression analysis elevated UAE was associated with significant stenoses (standardized adjusted odds ratio (OR) = 1.68 [1.15-2.44]; p=0.007). In this model, the categorical variable micralbuminuria exhibited an OR of 1.54 (1.03-2.30); p=0.034 and macroalbuminuria an OR of 2.55 (1.14-5.72); p=0.023) for significant stenoses. Similarily, the continuous variable ACR was significantly associated with significant coronary stenoses (OR=1.45 [1.13-1.86]; p=0.004). This association remained significant in the subgroups of patients with diabetes (1.66 [1.01-2.74]; p=0.045) and in patients without diabetes (OR=1.42 [1.05-1.92]; p=0.023).

Conclusion: An elevated UAE is strongly associated with angiographically determined coronary atherosclerosis both in patients with and without diabetes, independent of conventional cardiovascular risk factors and of the eGFR.

Poster Session

EVALUATION OF SYSTEMIC AND TISSUE-SPECIFIC INFLAMMATION AS RISK FACTOR FOR ATHEROSCLEROSIS IN PIGS FED WITH HIGH-FAT DIET

Roberto Giovannoni[1], M. Busnelli[1], M.G. Cerrito[1], A. Froio[1], S. Manzini[1], A. Vargiolu[1], B.E. Leone[1], M. Forni[2], M.L. Bacci[2], G.M. Biasi[1], M. Lavitrano[1]

[1]Surgical Sciences, University of Milano-Bicocca, Monza, [2]Veterinary Morphophysiology and Animal Production, University of Bologna, Ozzano dell'Emilia, Italy

Background and aims: It has been established that the inflammatory component represents a significant contribution in the background of clinically recognizable risk factors of atherosclerosis, for example, hypertension, hyperglycemia, dyslipidemia, obesity. The aim of this study was to evaluate systemic and tissue-specific inflammation in pigs fed with high-fat diet.

Methods: 21 pigs were kept for 16 weeks on a standard diet (SD; 3.6% lipids; n=7) or a high-fat diet (HFD; 22% lipids, 5% cholesterol; n=14). Seven HFD-fed pigs received high-dose atorvastatin (80 mg/die) starting after 8 weeks of hypercholesterolemic diet until the end of the experimental procedure.

Results: HFD administration significantly raised the number of circulating leukocytes, leading to a "hypercholesterolemia-associated monocytosis" and promoting the synthesis of several pro-inflammatory molecules. Hypercholesterolemia induced adipocytes hypertrophy and T-lymphocytes infiltration in abdominal white adipose tissue (WAT). HFD-fed pigs displayed liver inflammation, hepatic stellate cells activation with a concomitant increased infiltration of macrophages, T- and B-lymphocytes. Inflammation extended beyond liver and WAT with increased macrophage content in lung parenchyma. Atorvastatin abolished WAT inflammation by reducing adipocyte area and the number of infiltrating T-lymphocytes. In the liver, atorvastatin decreased hepatic stellate cells activation and the inflammatory infiltrate and lowered the amount of macrophages in lung parenchima of HFD-fed pigs.

Conclusion: Hypercholesterolemia significantly raised the amount of circulating leukocytes and exacerbated the inflammatory response in WAT, liver and lung. Atorvastatin treatment markedly decreased systemic and tissue-specific inflammatory markers by preventing the development of an inflammatory milieu and the accumulation of infiltrating leukocytes.

Poster Session

RELATION BETWEEN IRON STATUS PARAMETERS AND OTHER CARDIOVASCULAR RISK FACTORS EXTRACTED BY FACTOR ANALYSIS IN PATIENTS WITH CORONARY ARTERY DISEASE

Vesna Spasojevic-Kalimanovska[1], N. Bogavac-Stanojevic[1], S. Spasic[1], D. Kalimanovska-Ostric[2], J. Kotur-Stevuljevic[1], Z. Jelic-Ivanovic[1]

[1]Institute of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, [2]Institute for Cardiovascular Diseases, Clinical Center of Serbia, Belgrade, Serbia

Aims: Epidemiological evidence concerning the role of iron, a lipid peroxidation catalyst, in atherosclerosis and coronary artery disease (CAD) is inconsistent. Factor analysis was used to investigate the association of serum ferritin and soluble transferin receptors (sTfR) concentrations, as indicators of body iron status with the risk of CAD.

Methods: The study included 188 patients with angiographically approved CAD. Principal factor analysis was used to investigate clustering of variables associated with iron status in patients with CAD.

Results: Factor analysis resulted in a reduction of variable number from the original 15 to 5 composite clusters. All five factors had an eigenvalue ≥1 and they explained 64.44% of the total variance. These factors were:

1. “atherogenic” with positive loadings of TC, LDL-C, apoB and TG;
2. „antiatherogenic“ with positive loadings of HDL-C and apoA-I;
3. „inflammatory“ with positive loadings of hs-CRP, and fibrinogen;
4. „obesity“ with positive loadings of weigh and waist; and
5. „oxidative-stress status“ with positive loadings of SOD and age and negative loading of O2−.

In separate univariable analysis inflammatory factor predicted high ferritin (P=0.010), antiatherogenic factor predicted low ferritin (P = 0.002) and the atherogenic factor predicted high sTfR (P=0.006). In a multivariable model, including gender, each factor continued to be a significant predictor [inflammatory on ferritin OR=2.828 (1.175-6.809), P=0.020; antiatherogenic on ferritin OR=0.418 (0.189-0.925), P=0.031; atherogenic on sTfR OR=2.643 (1.326-5.269), P=0.006].

Conclusions: These data show an association between increased body iron stores and higher risk of CAD, confirming previous epidemiological findings.

Poster Session

CAROTID PLAQUES FROM SYMPTOMATIC STROKE PATIENTS SHOW INDUCTION OF GENES THAT ARE RELATED TO PROLIFERATION, ANGIOGENESIS AND NEURODEGENERATIVE DISORDERS

Raghu Vemuganti[1], R.J. Dempsey[1], K. Tureyen[1], S. Salamat[2], B. Hermann[3]

[1]Neurological Surgery, [2]Pathology & Laboratory Medicine, [3]Neurology, University of Wisconsin, Madison, WI, USA

In carotid atherosclerotic patients, asymptomatic plaques are more prevalent than symptomatic plaques. The mechanisms that induce a plaque to become symptomatic are not completely understood. In particular contribution of molecular factors for plaque maturation and rupture need to be analyzed. We currently used Affymetrix Human GeneChip to analyze expression profiles of ~45,000 mRNA transcripts in surgically removed carotid artery plaques from 6 symptomatic stroke patients and 4 asymptomatic patients. Hierarchical clustering analysis revealed a statistically significant increase in the expression of 236 genes (>2 fold, $p < 0.05$ in each case) in the symptomatic over the asymptomatic plaques. Pathways analysis revealed that cell proliferation/differentiation is the most predominant pathway in the symptomatic plaques. Furthermore, many genes increased in the symptomatic plaques are related to degenerative disorders and angiogenesis. In a separate cohort of endarterectomy patients, baseline cognitive testing revealed abnormal mental status with difficulty in immediate memory, visuoconstructional skills and attention. This suggests the possibility that symptomatic atherosclerotic disease might be related to later neurodegenerative disorders. Histopathological analysis revealed higher number of newly formed capillaries in the symptomatic plaques that might contribute to their rupture. Thus, these studies suggest a molecular evidence of increased risk of neurodegeneration, neoplasia and angiogenesis in the subset of carotid atherosclerotic patients that suffer plaque embolisation and stroke. Furthermore, atherosclerotic plaques can be different at the molecular level between symptomatic and asymptomatic patients. The implications of these findings suggest that both prediction and prevention of stroke symptoms may become possible at the genetic level.

Poster Session

IL-6 SERUM LEVELS AND PRODUCTION IN PCOS ADOLESCENTS IS RELATED TO INSULIN RESISTANCE

Sabrina Uda[1], A.M. Fulghesu[2], F. Sanna[1], R. Magnini[2], E. Portoghese[2], S. Accossu[1], P.P. Contini[3], B. Batetta[1]

[1]Dipartimento di Scienze e Tecnologie Biomediche, [2]Dipartimento Materno Infantile e di Scienze delle Immagini, University of Cagliari, [3]Centro Malattie Dismetaboliche S. Giovanni di Dio, Azienda Universitaria Ospedaliera, Cagliari, Italy

Polycystic ovarian syndrome (PCOS) is an endocrine disorder frequently characterised by obesity and metabolic diseases including hypertension, insulin resistance and diabetes in adulthood, all leading to an increased risk of atherosclerosis.

The present study aimed to evaluate serum and production of inflammatory markers in insulin and non insulin-resistant adolescent Sardinian PCOS.

On the basis of HOMA findings, patients were divided into two groups: non insulinresistant (NIR) and insulinresistant (IR), and were weight- and age-matched with healthy girls. Serum inflammatory cytokines (TNF-α, IL-6, Il-10, TGF-β) and *in vitro* inflammatory lympho-monocyte response to microbial and mitogenic stimuli.

Although serum levels of cytokines were similar in all groups Interleukin-6 (IL-6) was significantly higher in IR PCOS. Moreover, in the latter group lipopolysaccharide-activated monocytes secreted significantly higher levels of IL-6 compared to NIR and control subjects. IR PCOS displayed increased IL-6 serum levels and higher secretion in LPS-activated monocytes, whilst revealing no differences for other inflammatory cytokines. The results obtained suggest that in PCOS patients an altered IL-6 response to inflammatory stimuli is present in IR, likely contributing towards determining onset of a low grade inflammation.

Poster Session

APOLIPOPROTEIN E GENOTYPE, CARDIOVASCULAR BIOMARKERS AND STROKE RISK: SYSTEMATIC REVIEW OF 10,550 STROKE CASES AND POOLED ANALYSIS OF 74,052 INDIVIDUALS

Tina Shah[1], T. Khan[2], A. Hingorani[1], J. Casas[2], APOE Collaboration

[1]University College London, [2]London School of Hygiene and Tropical Medicine, London, UK

Introduction: The ε2/ε3/ε4 variants in apolipoprotein E (*APOE*) exhibit a positive linear association with LDL-cholesterol and CHD risk. However, the association of *APOE* genotype with other cardiovascular biomarkers and risk of ischaemic stroke is less well defined.

Objectives: We evaluated the association of *APOE* genotype with risk of ischaemic stroke and assessed consistency with the effects of *APOE* genotype on LDL-cholesterol or other lipids and biomarkers of cardiovascular risk.

Methods: We conducted a systematic review of studies reporting on *APOE* genotype and ischaemic stroke and a pooled analysis of primary data on *APOE* genotype, lipids, other circulating biomarkers of cardiovascular risk, and carotid intima-media thickness (CIMT).

Results: In 70 studies (10,785 cases, 61,674 healthy individuals), the association of *APOE* genotype with ischaemic stroke was non-linear. The odds ratios with ε3/ε3 as reference were ε2/ε2: 1.44 (95%CI 1.12-1.84); ε2/ε3: 0.94 (0.84-1.05); ε2/ε4: 1.25 (1.08-1.48); ε3/ε4: 1.28 (1.12-1.45); ε4/ε4: 1.48 (1.17-1.88). In 14 studies (upto 74,052 individuals), *APOE* genotype was linearly and positively associated with LDL-cholesterol, apoB and CIMT, and negatively and linearly with apoE, and HDL-cholesterol. The associations with Lp(a), CRP and triglycerides were non-linear, and the shape of the association with triglycerides most closely resembled the association with ischemic stroke.

Conclusions: The non-linear association of *APOE* genotype with ischaemic stroke, in contrast to its linear positive association with LDL-cholesterol, CIMT and CHD risk suggests that the stroke association may be mediated through risk factors other than LDL-cholesterol, with triglycerides being one promising candidate.

Poster Session

SOCIAL BUT NOT PHYSICAL STRESSORS RESULTS IN IL-6 RELEASE IN MICE

Evelina Bernberg[1], M.E. Johansson[2], G. Bergström[1]

[1]The Wallenberg Laboratory, Dept of Molecular and Clinical Medicine/Clinical Physiology, [2]Dept of Physiology, Inst of Neuroscience and Physiology, The Sahlgrenska Academy at The University of Gothenburg, Göteborg, Sweden

Introduction: We have shown that different forms of stress affect atherogenesis in mice differently. To unravel the mechanism behind this difference we compared the effect of five physical stressors with a social form of stress on systemic release of corticosterone and interleukin-6 (IL-6).

Methods: Male ApoE$^{-/-}$ or C57BL/6 mice were exposed to social disruption stress (SDR; 2 h for 4 days). Further, SDR was compared with five physical stressors (Air-jet, Balance, Restraint, Rat odor, Restraint + rat odor). A β_1-adrenoceptor antagonist (metoprolol) was used to evaluate the effect of sympathetic nervous system activity on IL-6 release during SDR. Plasma IL-6 and corticosterone levels were analyzed immediately after stress.

Results: SDR increased corticosterone and IL-6 levels in plasma of C57BL/6 mice (SDR vs. control, $p< 0.01$ for corticosterone, $p< 0.001$ for IL-6). Moreover, SDR increased plasma IL-6 levels in ApoE$^{-/-}$ mice ($p< 0.05$ for SDR vs. control), but this effect could not be blocked by metoprolol. Plasma corticosterone levels were increased for all physical stressors ($p< 0.01$ vs. control). Interestingly, no effect on plasma IL-6 was seen after exposure to four of the physical stressors, only restraint + rat odor increased plasma IL-6 levels ($p< 0.01$ vs. control).

Conclusions: These findings suggest that SDR leads to IL-6 release not observed after exposure to other forms of stress. Further, IL-6 production after SDR does not seem to be mediated via β_1-adrenoceptors. The observed differential stress-response may be an explanation to the observation that social but not physical stress results in increased atherosclerosis.

Poster Session

LOW ARYLESTERASE ACTIVITY AND R ALLELE (PON1 Q192R POLYMORPHISM) ARE THE PUTATIVE RISK FACTORS OF CAD

Imteyaz Ahmad[1], R. Narang[2], A. Venkatraman[1], N. Das[1]

[1]Biochemistry, [2]Cardiology, All India Institute of Medical Sciences, New Delhi, India

The present study aimed at monitoring the activity of paraoxonase/arylesterase activity in patients with CAD, and the frequency distribution of PON1 Q192R and L55M polymorphisms in the residents of Delhi and surrounding areas. Simultaneously, the lipid profiles of the study subjects were studied. The study population consisted of 195 angiographically CAD Patients and 140 control participants. Serum lipids were measured by standard methods, paraoxonase/arylesterase activities by spectrophotometric assay of p-nitrophenol/phenol production following addition of paraoxon/phenylacetate to serum. PON1 L55M and Q192R genotypes were determined by PCR-RFLP. A decline by 17.42% arylesterase activity (85.08±39.47 vs. controls 103.03±46.88, P=0.048) in patients. The genotype distribution of Q192R in the controls were QQ (46.63%), QR (39.33%) and RR (14.04%) and in the patients were QQ (31.86%), QR (50.49%) and RR (17.65%). R allele was more frequent in patients than the controls (0.43 versus 0.34; P=0.013). The genotype distribution of PON1 L55M in the controls were LL (71.91%), LM (26.97%) and MM (1.12%) and in the patients were LL (64.71%), LM (32.84%) and MM (2.45%). Hardy-Weinberg equilibrium was obeyed. The odds ratio for developing CAD with the R allele (RR+QR genotype) was 1.86 (95%CI 1.23 to 2.83, P=0.003) compared with Q allele (QQ genotype). Patients showed significantly higher levels of LDL-c, LDL-c/HDL-c ratio and lower levels of HDL-c than the controls. To conclude, individuals with lower than normal levels of HDL-c, arylesterase activity and R allele may be at a higher risk of developing CAD.

Poster Session

THE RESTING HEART RATE STRONGLY PREDICTS ALL-CAUSE DEATH AMONG MIDDLE AGED WOMEN AND MEN FROM A GENERAL POPULATION

G. Nilsson, **Andreas Rosenblad**, J. Leppert

Center for Clinical Research Västerås, Uppsala University, Västerås, Sweden

Introduction: The resting heart rate (HR) has been found to predict all-cause death among middle aged healthy men. Corresponding data for women are lacking. We report the sex specific predictive ability of heart rate for all-cause mortality among middle aged women and men.

Material and methods: Women and men from the county of Västmanland, Sweden (260000 inhabitants) were invited to a health survey at the age of 40 and 50 during the years 1990-99 (participation rate 48%). HR was measured as radial pulse rate. Relative risk (RR) for all-cause death was computed by Cox regression (follow-up until 2008-08-17).

Results: Among 15965 men and 17250 women, 751 men and 537 women died during follow-up (deaths per person-year: men 0.34%, women 0.22%). The mean HR was 69 beats per minute (bpm) for men and 71 for women. The RR, (95% confidence interval) for 1 bpm increase of HR was 1.034 (1.027-1.041); p>0.001 for men and 1.029 (1.021-1.038); p>0.001 for women. Multivariable adjustment (prevalent smoking, diabetes, hypertension, myocardial infarction, stroke as well as cholesterol, BMI, systolic blood pressure and age cohort) attenuated the RR; men 1.023 (1.016-1.031); p>0.001 and women 1.021 (1.013-1.030); p>0.001.

The RR for people within the upper quartile (pulse rate>76) compared with those within the lowest quartile (pulse rate < 65) was 2.3 (1.9-2.8) for men and 1.9 (1.5-2.4) for women.

Conclusion: HR is a strong predictor of all-cause death among middle-aged women and men.

Poster Session

THE ASSOCIATION BETWEEN D-VITAMIN STATUS, GLUCOSE AND METABOLIC SYNDROM IN GENERAL POPULATION

Otto Mayer[1], J. Vrzalová[2]

[1]2nd Dept. of Internal Medicine, [2]Dept. of Immunodiagnostics, University Hospital and Medical Faculty, Charles University, Plzen, Czech Republic

Background: There is an accumulating evidence, that D vitamin status plays a role not only in context of bone health, but also in several adverse health condition. We aimed to establish the association between mild decrease of serum monohydroxyde D-vitamin concentration ($_{25}$OH-D) and some glucose metabolism parameters.

Methods: 576 subjects (mean age 48.03y (SD 14.8)), 41.5% males), a Pilsen sub-sample of postMONICA survey were included into cross-sectional study. 25(OH)-D was estimated by RIA method, metabolic syndrom by current NCEP-ATIII definition.

Results: We found a negative trend between D vitamin and several glucose metabolism parameters. With increasing $_{25}$OH-D in quartiles, the serum fasting glucose (5.52±0.11, 5.50±0.11, 5.18±0.06, 5.04±0.05 mmol/L, resp., $p < 0.0004$), prevalence of overt diabetes (10.1, 7.5, 4.2, 1.4 %, resp., $p < 0.006$) and of metabolic syndrom (27.6, 26.8 ,18.3, 12.2 % resp., $p< 0.0008$) significantly decreased. All these associations remained significant, even after adjustement for age, gender and other conventional risk factor as potential confounders.

Conclusion: In our sample of general population we found a clear significant and independent negative association between D vitamin several glucose metabolism disorders.

(Supported by Internal Grant Agency of Ministry of Health, CZ).

Poster Session

DOES INSULIN RESISTANCE INFLUENCE ENDOTHELIAL PROGENITOR CELLS IN OBESE PATIENTS?

E. Miller-Kasprzak[1], **Pawel Bogdanski**[2], D. Pupek-Musialik[2], P.P. Jagodzinski[1]

[1]Department of Biochemistry and Molecular Biology, [2]Department of Internal Medicine, Metabolic Disorders and Hypertension, University of Medical Sciences, Poznan, Poland

Objective: To investigate the relation between endothelial progenitor cells (EPC) and insulin resistance in obese individuals.

Methods: Thirty-eight obese adults (aged 42.5±12.7), body mass index (BMI) (32.3±4.0) and 13 normal weight controls (aged 48.2±12.9; BMI 23.2±2.3) were included into the study. Peripheral blood (PB) mononuclear cells were separated by ficoll gradient centrifugation and cultured in five-day colony forming unit-endothelial cells (CFU-EC) assay to develop EPC. Insulin resistance in examined subjects was evaluated according to homeostasis model assessment-insulin resistance (HOMA-IR) protocol.

Results: Decreased number of EPC in studied group was found as compared to healthy control ($p=0.001$). The inverse correlation between HOMA-IR index and EPC number ($r=-0.558$, $p< 0.0001$) was noticed. Additionally, correlations between triglyceride (TG) concentration, high density lipoproteins (HDL) concentration, body composition parameters in obese the subjects were established.

Conclusion: Our results show that insulin resistance and have a significant impact on the EPC in obesity. Decreased number of EPC should be analyzed as an important factor in the complex pathogenesis of endothelium dysfunction observed in obesity.

This work was supported by grant no. NN 402 253934 (2008-2010) from the Polish Ministry of Science and Higher Education

Poster Session

HIGH LIPOPROTEIN(A) LEVEL IS ASSOCIATED WITH POOR LONG-TERM PROGNOSIS AFTER CORONARY ARTERY BYPASS GRAFTING

Marat Ezhov[1], O. Afanasieva[2], A. Lyakishev[1], M. Afanasieva[2], R. Akchurin[3], S. Pokrovsky[2]

[1]Problems of Atherosclerosis, [2]Laboratory of Problems of Atherosclerosis, [3]Cardiology Research Center, Moscow, Russia

The aim of the present prospective study is to evaluate whether lipoprotein(a) [Lp(a)] concentration is a predictor of long-term adverse outcomes in patients after coronary artery bypass grafting (CABG).

Methods: We followed 263 patients with coronary heart disease (233 men, 30 women, mean age 55±9 years) for a mean time 69±44 months who underwent the operation of coronary artery bypass grafting. Before the surgery blood samples were collected and lipid profile and Lp(a) concentrations were measured in all patients. The main end-points were cardiac death and nonfatal myocardial infarction, hospitalization for recurrent angina and repeat coronary revascularization.

Results: Total number of coronary outcomes was 122, including death - 20 (16%), myocardial infarction - 14 (11%), percutaneous coronary interventions - 29 (24%), re-CABG - 6 (5%) and recurrent angina - 53 (43%). The incidence of death was 11.3% (11 of 93) in patients with Lp(a) $\geq$ 30 mg/dL and 5.2% (9 of 170) in those with Lp(a) < 30 mg/dL. Lp(a) level was an independent predictor of combined end-point (hazard ratio 3.24; 95% confidence interval 2.18-4.83, p< 0.001), cardiac death (2.89; 1.31-6.35, p< 0.01) and cardiac death plus nonfatal myocardial infarction (2.95; 1.50-5.79, p< 0.001). Other independent predictors of main outcomes were diabetes mellitus (2.89; 1.69-4.92, p< 0.001) and triglycerides levels (1.16; 1.02-1.32, p< 0.05). Use of statins was associated with decreased risk of cardiac death: 0.33; 0.15-0.76, p< 0.01.

Conclusion: Elevated levels of Lp(a) is a predictor of late coronary events including death and nonfatal myocardial infarction after CABG.

Poster Session

ASSOCIATION OF ELEVATED LIPOPROTEIN(A) LEVEL WITH PREMATURE CORONARY HEART DISEASE IN MEN AND WOMEN

M. Safarova, **Marat Ezhov**, O. Afanasieva, A. Lyakishev, S. Pokrovsky

Cardiology Research Center, Moscow, Russia

Objective: To assess the relationship of lipoprotein(a) [Lp(a)] concentration with clinical manifestation of coronary heart disease (CHD) in younger age.

Methods: In this cross-sectional study we recruited 1483 patients (85% male) with CHD verified by angiography. In accordance with the level of Lp(a), patients were divided into 2 main groups: I (n = 599) ≥ 30 mg/dL, and II (n = 884) < 30 mg/dL. The following risk factors were recorded: hypertension, smoking, diabetes mellitus, obesity, and family history of CHD. Lipid profile, Lp(a), hsCRP concentrations were measured in all patients.

Results: Among 496 men (mean age 53.7 ± 9.5 years) from Group I, age of CHD manifestation was 49.8 ± 8.8 years vs. 51.0 ± 9.0 years in 767 men from Group II (mean age 54.6 ± 9.2 years), p< 0.05. In women from Group I (n = 103, mean age 56,8 ± 8,4 years) age of CHD onset, was 53.1 ± 8.3 years vs. 55.8 ± 10.0 years in Group II, p < 0.05. There was no significant difference in the classic risk factors, hsCRP and fibrinogen levels between the groups. The incidence of CHD in men younger than 45 years was 36% in Group I and 28% in Group II, p< 0.01. The incidence of CHD in women before 55 years was 66% in Group I and 47% in parallel group, p< 0.01.

Conclusion: Our data suggest that elevated Lp(a) is independently associated with the development of early CHD in men as well as in women.

Poster Session

AORTIC BIFURCATION ANGLE AS AN INDEPENDENT RISK FACTOR FOR AORTOILIAC OCCLUSIVE DISEASE

Abolhassan Shakeri Bavil[1], R.S. Tubbs[2], M.M. Shoja[1], H. Nosratinia[1], W.J. Oakes[3], M. Nemati[1]

[1]Tabriz University of Medical Sciences, Tabriz, Iran, [2]University of Alabama at Birmingham, Birmingham, AL, USA, [3]St George's University, West Indies, Grenada

Introduction: Recently, there has been interest in potential geometric risk factors that might result in or exaggerate atherosclerosis. The aortic bifurcation is a complex anatomical area dividing the high pressure blood of the descending abdominal aorta into the lower limbs and pelvis. The distribution of the bifurcation angle and any asymmetry, its relation with age and its possible contribution to the risk of aortoiliac atherosclerosis are presented here.

Materials and methods: Statistical analysis was performed by SPSS version 11.0 using, Fisher`s exact test, the Pearson and Spearman correlation tests and logistic regression analysis. The p value was set at 0.05.

Results: No correlations were found between age, bifurcation angle and angle asymmetry in the Pearson test ($p > 0.05$). Logistic regression analysis revealed that the bifurcation angle, but not its asymmetry, gender or age, was a significant and independent risk factor for aortoiliac atherosclerosis (model $r2 = 0.662$, $p = 0.027$). With additional study these results may have implications regarding risk factors for aortoiliac atherosclerosis.

Conclusion: To our knowledge, this study is the first of its kind to indicate the potential of such an important geometric risk factor for atherosclerosis at the aortic bifurcation.

Poster Session

PREVALENCE OF THE CLASSICAL CARDIOVASCULAR RISK FACTORS IN ROMA MINORITY POPULATION OF CROATIA

Hrvojka Marija Zeljko[1], T. Škarić-Juric[2], N. Smolej Narancic[2], M. Pericic Salihovic[2], I. Martinovic Klaric[2], J. Milicic[2], M. Barbalic[2], M. Zajc[2], L. Barac Lauc[2], B. Janicijevic[2]

[1]Internal Medicine, GH 'Sv. Duh', University of Zagreb, [2]Institute for Anthropological Research, Zagreb, Croatia

Purpose: Roma are an ethnic minority of northern Indian origin. They are well known for preserved traditions and resistance to assimilation and therefore usually marginalized economically, spatially, politically and in terms of culture. Since the current knowledge about the prevention of cardiovascular diseases in Roma population is scarce, the objective of the study is to assess the prevalence of cardiovascular diseases' risk factors.

Methods: A multidisciplinary community-based study was carried out in rural areas of two regions of Croatia - Baranja and Međimurje. The sample encompassed a total of 423 randomly selected members (144 men and 279 women) of the Bayash Roma minority population aged 18-84 (41.1 ± 15.2). The population prevalence of hypertension was assessed as SBP ≥ 140 or DBP ≥ 90 mmHg or taking antihypertensive therapy. Overweight was assessed BMI ≥ 25 (kg/m^2). Smoking status was assessed within the course of the interview held by experienced medical staff. Blood pressure measurements and short anthropometrics were carried out using standard protocols. The lipid status and fasting glucose was determined from blood samples and standard cut-offs were used.

Conclusions: Although the overall prevalence of hypertension in Roma was almost half the magnitude usually reported for general population of Croatia, the presented results have shown that this population bears a high CVD risk factors load. It is related to smoking, obesity, and high glucose and lipids levels. We expect that, along with westernization of their life-style and increase of their economic power, the proportion of CVD will also increase.

Poster Session

PREDICTION OFR THE POPULATION AT RISK OF ATHEROTHROMBOTIC DISEASE IS INDEPENDENT OF BLOOD SUGAR LEVEL

William Feeman, Jr

The Bowling Green Study, Bowling Green, OH, USA

Introduction: Diabetes is considered to be a "CHD equivalent." However the risk of atherothrombotic disease (ATD) is not equal for all diabetics and milder elevations of blood sugar are not in and of themselves causal for ATD. It is important to define the ATD risk, if any, in patients with blood sugar level elevations.

Objective: The purpose of this submission is to show that the prediction of the population at risk of ATD is essentially independent of blod sugar level.

Materials and methods: The Bowling Green Study (BGS) has developed a multi-factorial risk factor graph for the prediction of the population at risk of ATD, utilizing the Cholesterol Retention Fraction (CRF, or [LDL-HDL]/LDL) on the ordinant and Systolic Blood Pressure (SBP) on the ordinant. A threshold line can be delineated separating the mainstream of ATD patients from a few outliers. The line coordinants of the CRF-SBP plots are (o.74,100) and (0.59,140).

Results: 85% of all ATD patients in the author´s database have CRF-SBP plots abaove the threshold line. Those ATD patients with CRF-SBP plots below the threshold line are mainly cigarette smokers, leaving only 6% of the ATD population that can not be predicted by CRF-SBP plot position above the threshold line and/or cigarette smoking status. These findings are independent of blood sugar level.

Conclusions: The population at risk of ATD cam be accurately predicted independently of blood sugar level.

Poster Session

EFFECTS OF RAMADAN FASTING ON CARDIOVASCULAR RISK FACTORS

A. Norouzy, **Mohsen Nematy**, M. Alinezhad-Namaghi, M. Shakhskalaee, M. Mozhdehifard, S. Sajjadi, M. Sabery

Dept. of Biochemisrty&Nutrition, Mashad Medical School, Mashad, Iran

Introduction: Ramadan is a month for Muslims during which they abstain from eating, drinking and smoking from dawn to sunset. The recommendations regarding the safety of fasting for patients with a previous cardio/cerebrovascular event have been based on expert opinion rather than evidence based medicine and sometimes controversial. In the present study, the effect of Ramadan fasting on some cardiovascular risk factors has been investigated.

Methods: 82 volunteers (38 male and 44 female, age 54±10 years, fasted for at least 10 days) with a previous history of ischaemic vascular event were recruited. A fasting blood sample was obtained and blood pressure, waist circumference and body mass index (BMI) were measured at the beginning and at the end of month of Ramadan (BR&ER respectively). Plasma lipids profile, glucose, insulin, HsCRP and homocysteine were analyzed.

Results: There was a significant higher HDL (BR:43±9, ER:48±8mg/dl) and lower HsCRP (BR:2.3±3, ER:1.9±3mg/l), Cholesterol (BR:193±51, ER:184±42mg/dl), TG (BR:225±129, ER:183±112mg/dl), LDL (BR:110±41, ER:97±32 mg/dl), VLDL (BR:38±14, ER:34±17 mg/dl), systolic blood pressure (BR:133±16, ER:130±17mmHg) and body mass index (BR:28.4±4, ER:27.7±4kg/m^2) after Ramadan ($p< 0.05$, t-test).The changes in plasma glucose (BR:107±11, ER:106±14mg/dl), HOMA-IR (BR:3.8 ±3, ER:3.6 ±3), homocysteine (BR:10.5±4, ER:11.5 ±4µmol/l) and waist circumference (BR:98±10, ER:97±10cm) was not significant ($p>0.05$, t-test).

Conclusion: This study shows there is a significant improvement in some cardiovascular risks after fasting in month of Ramadan. However a study with bigger sample size, other habitual changes controlled and with a control group is needed.

Poster Session

PLA2G2A, *PLA2G5* AND *PLA2G10* VARIANTS, SPLA2 ACTIVITY AND MASS AND CHD RISK: RESULTS FROM GRACE AND EPIC-NORFOLK

M. Guardiola[1], H.J. Exeter[1], J. Palmen[1], C. Perret[2], K. Li[1], J.A. Cooper[1], K.-T. Khaw[3], M.S. Sandhu[3], S. Ricketts[3], A. Tedgui[4], S.E. Humphries[1], E. Ninio[2], S.-A. Karabina[2], S.M. Boekhold[5], Z. Mallat[4], **Philippa J Talmud**[1]

[1]Division of Cardiovascular Genetics, Department of Medicine, University College of London, London, UK, [2]INSERM UMRS 937, Université Pierre et Marie Curie UPMC-Paris 6, Faculté de Médecine Pierre et Marie Curie, Paris, France, [3]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge, UK, [4]INSERM, U689, Centre de Recherche Cardiovasculaire Lariboisiere, Paris, France, [5]Department of Cardiology, Academic Medical Center, Amsterdam, The Netherlands

Background: Plasma secretory (s)PLA2 levels and activity are associated with CHD risk with activity having the greater prognostic value. While activity is thought to be a composite of sPLA2-IIa, -V and -X, mass is sPLA2-IIa specific. We examined the contribution of variants of genes encoding sPLA2s, *PLA2G2A, PLA2G5, PLA2G10,* with sPLA2 activity and mass and CHD risk in GRACE, a study of acute coronary syndromes (n=347) and a cohort from the EPIC-Norfolk study (1138 cases: 2237controls).

Results: Six SNPs in *PLA2G2A,* 5 in *PLA2G5* and 4 in *PLA2G10* were genotyped. Considering activity, only *PLA2G2A* rs11573156 showed a significant effect (+5% $p=2x10^{-4}$) in EPIC-Norfolk with a stronger raising-effect in GRACE (+54% p=0.07). The functional *PLA2G10* R38C was associated with -35% lower activity (p=0.016) in GRACE. Four *PLA2G2A* SNPs showed strong association with sPLA2-IIa levels in both studies with upto 135% difference between common and rare homozygotes ($p=7.82x10^{-193}$) for rs11573156 in EPIC-Norfolk. After stepwise regression analysis, in EPIC-Norfolk *PLA2G2A* rs11573156 predicted activity and with rs3767221 independently predicted mass. In GRACE *PLA2G10* R38C alone predicted activity. *PLA2G2A* rs11573156 was associated with increased CHD risk (OR1.64 95%CI 1.18-2.28).

Conclusion: Variants in *PLA2G2A* and *PLA2G10* used as instrumental variables, identified these genes as contributing to sPLA2 activity while *PLA2G2A* alone was the strong determinant of sPLA2 mass. The participation of *PLAG10* in sPLA2 activity needs replication whereas *PLA2G5* does not contribute directly to activity or mass. The activity- and mass-raising *PLA2G2A* rs11573156 showed CHD risk association, suggesting a causal relationship between *PLA2G2A* and CHD.

Poster Session

PLASMA OXIDIZED LDL AND THIOL-CONTAINING MOLECULES IN PATIENTS WITH EXUDATIVE AGE-RELATED MACULAR DEGENERATION

Amir Ghorbanihaghjo[1], A. Javadzadeh[2], E. Bahreini[1], N. Rashtchizadeh[2], S. Alizadeh[2]

[1]Biochemistry Department and Biotechnology Research Center, [2]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Purpose: Plasma oxidized LDL (OX-LDL) concentration has been reported to be a risk factor for atherosclerotic vascular disease. We assessed the plasma OX-LDL and thiol compound content concentrations in Exudative Age Related Macular Degeneration (E-ARMD), a known atherosclerotic process.

Methods: Forty-five patients with E-ARMD were compared with 45 sex and age matched healthy controls. Plasma levels of Hcy, total thiols (tSH), glutathione (GSH) and OX-LDL were measured in both of the groups.

Results: Total Hcy (15.4 ± 7.2 µM vs. 10.7 ± 3.7 µM ; $P < 0.001$) and OX-LDL (52.2 ± 13.8 mU/L vs. 37.8 ± 10.8 mU/L; $P < 0.001$) levels were significantly higher, while GSH (1.10 ± 0.97 µM vs. 2.09 ± 1.04 µM; $P < 0.001$) and tSH (0.31 ± 0.06 mg/dl vs. 0.35 ± 0.05 mg/dl; $P < 0.001$) were markedly lower in the patients with E-ARMD than in the control group, respectively. The plasma OX-LDL concentration also exhibited a positive and significant correlation with Hcy ($r = 0.719$, $P < 0.001$) in patients with E-ARMD.

Conclusions: Increased concentrations of oxidative and decreased concentrations of antioxidative markers suggest that oxidative stress may play a role in inducing E- ARMD.

Poster Session

VASCULAR DYSFUNCTION IN ESRD PATIENTS UNDER REPLACEMENT THERAPY

Saeed Abdelwhab

Faculty of Medicine, Ain Shams University, Cairo, Egypt

Background: Endothelial dysfunction (ED) is an important factor in the pathogenesis of atherosclerosis, which is the major cause of mortality and morbidity in ESRD patients.

Methods: This study 25 ESRD patients on regular hemodialysis (HD) (group 1), 15 ESRD patients on intermittent peritoneal dialysis (PD) (group 2) and 20 healthy control subjects (group 3). For all groups complete medical history and thorough examination, biochemical Laboratory tests including renal function tests, calcium, phosphorus, intact PTH and Echocardiography. Endothelial function was assessed by Flow mediated dilatation of the brachial artery (FMD) evaluated non-invasively by B-mode ultrasonography.

Results: Comparison of FMD of brachial artery shows significant difference between group one and control ($p< 0.001$), group two and control group ($p< 0.001$), and between group 1 and 2 ($p< 0.001$). Multiple regression test for brachial artery flow mediated dilatation shows significant correlations with age ($p< 0001$), serum LDL ($p=0.04$), hematocrit value ($p< 0.001$), serum PTH ($P=0.01$) and presence of DM ($p=0.009$). No correlation is found with duration of dialysis ($p=0.57$), total cholesterol ($p=0.62$), serum triglyceride ($p=0.10$) nor with calcium phosphorus products ($p=0.08$).

Conclusion: Endothelial function is impaired in ESRD patients than normal control subjects. PD patients had more endothelial dysfunction when compared to HD patients. ED is independently affected by age, LDL, PTH, hematocrit, and presence of diabetes mellitus. Hematocrit, PTH, and LDL are the main correctable factors affecting ED.

Poster Session

CRITICAL ISCHAEMIA AND MRF SCORE OF ATHEROSCLEROTIC DISEASE

M. Dilic[1], **Emir Kurtalic**[2], A. Begic[2]

[1]Clinical Center Sarajevo, [2]Angiology, Clinical Center Sarajevo, Sarajevo, Bosnia-Herzegovina

Introduction: Atherosclerotic disease is the leading cause of morbidity and mortality, especially in older populations. Most often, its presents as coronary, cerebrovascular or limb artery occlusion. Aim of this retrospective study was to analyze an MRF scor in patients treated at the Institute of vascular dieseases in period december 2007-december 2008 for terminal limb ischaemia.

Materials and methods: 63 patients with single acute, repeated acute or chronic limb ischaemia were included. We estimated several paramethers: sex; age, smoking, arterial hypertension, hyperlipoproteinemia, diabetes, increased level of fibrinogen and obesity (defined as BMI over 30). MRF scor was calculated as x/8. We classified the patients according to the level of necrotic demarcation as pedal, crural, femoral level and correlated it with calculated MRF.

Results: Patients with single episode of acute limb ischaemia, with repeated episodes of acute ischaemia and pts. with chronic terminal ischaemia have had an average MRF score of 5.11, 5.85 and 6.62 respectively. Patients with pedal, crural and femoral level of demarcation have had a MRF score of 5.6, 5.62 and 4.84 respectively.

Conclusion: MRF score of patients with chronic terminal limb ischaemia was signifikantnly higher ($p< 0,001$) then MRF score of patients with single episode or repeated episode of acute ischaemia. Patients with crural level of ischaemic demarcation have had highest MRF score compared with pedal and femoral ischaemia but the difference was not statistically significant ($p>0,05$).

Keywords: MRF, terminal limb ischaemia.

Poster Session

THE PREVELANCE OF SUBCLINICAL HYPOTHYROIDISM AMONG PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

Ertugrul Okuyan[1], A. Uslu[2], O. Levent[2]

[1]Bagcilar Education and Research Hospital, Istanbul, [2]Serife Baci State Hospital, Kastamonu, Turkey

Previous studies have suggested that thyroid dysfunction, as manifested by abnormalities in thyroid-stimulating hormone (TSH) levels, are associated with detrimental effects on the cardiovascular system. We aimed to evaluate the prevelance of subclinical hypothyroidism in 315 patients with the diagnosis of acute myocardial infarction (AMI).

We evaluate free T3, free T4 and TSH levels of 315 patients (Age 57.3±13) retrospectively, who have been admitted to a coronary intensive care unit between years 2004-2006 Turkey with the diagnosis of both ST and Non-ST elevation AMI. 197 patients were male and 118 were female. After the exclusion of patients with abnormal thyroxine levels, subclinical hypothyroidism was defined as a TSH level of 4.5 mIU/L or greater. According to this criteria, 31 of 197(15.73%) male and 17 of 118(14.40%) female patients had subclinical hypothyroidism(Total 48 of 315 patients %15.23).

The rate of subclinical hypothyroidism in our study population was %15.23. Actually it is a frequent pathology as its prevalance ranges from 1% among young adults to 10% beyond 60 years of age. The increased frequency in our AMI patients can represent potential cardiovascular risks of subclinical hypothyroidism.

Poster Session

PLASMA INFLAMMATORY MARKERS AND SUBCLINICAL ATHEROSCLEROSIS

Andrie Panayiotou[1,2], M. Griffin[3], N. Georgiou[1], D. Bond[3], D. Hoppensteadt[4], J. Fareed[4], A. Nicolaides[1,5]

[1]Cyprus Cardiovascular Disease Educational and Research Trust, Nicosia, Cyprus, [2]The London School of Hygiene and Tropical Medicine, [3]Vascular Screening and DIagnostic Centre, London, UK, [4]Loyola University, Chicago, IL, USA, [5]Imperial College, London, UK

Fibrinogen (Fb), high-sensitivity CRP (hsCRP), interleukin-6 (IL6), myeloperoxidide (MPO) and monocyte chemoattractant protein 1 (MCP1) are inflammatory markers, elevated in the presence of clinical atherosclerotic disease. The aim of the study was to determine whether this is also true for subclinical atherosclerosis.

Both common carotid and femoral bifurcations were scanned with ultrasound in 767 volunteers over 40. Four ultrasonic features were considered: (a) IMTcc, (b) carotid total plaque area, (c) femoral total plaque area and (d) total plaque area in all four arteries scanned. Plasma Fb, hsCRP, IL6, MPO and MCP1 levels were determined and their association with quartiles of the ultrasonic measurements were determined. P for trend was calculated.

Fb was associated with IMTcc and all plaque area measurements ($p < 0.01$). CRP and MPO were associated with IMTcc measurements ($p < 0.01$) but not plaque area measurements. IL6 was associated with all plaque area measurements ($p < 0.02$) but not IMTcc measurements. MCP1 was associated with carotid plaque area ($p < 0.01$) but not with IMT or Femoral plaque area and had a borderline association with total plaque area.

The findings support the hypothesis that IMTcc and plaque size have different biological determinants and are different expressions of subclinical atherosclerosis.

Poster Session

PREVALENCE OF LOW HDL-C IN STATIN TREATED PATIENTS THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], E. Alegria[3], C. Guijarro[4], B. Gonzalez-Timon[5], G. Vitale[5]

[1]Hospital Gregorio Marañon, Madrid, [2]Hopital Clinco Universitario, Santiago de Compostela, Santiago de Compostela (La Coruña), [3]Clinica Universitaria de Pamplona, Pamplona (Navarra), [4]Hospital de Alcorcon, Alcorcon (Madrid), [5]Merck & Co., MSD, Madrid, Spain

Introduction: Although statins reduce cardiovascular morbidity and mortality,the incidence of cardiovascular events is still high.Some studies have found a relationship between low levels of HDL-C and cardiovascular (CV) episodes even in patients on intensive statin therapy with LDL-C at goal. In this analysis we assess the prevalence of low HDL-C in statin treated Spanish patients included in DYSIS.

Methods: Analysis of 3710 Spanish patients included in DYSIS (22063 patients in Europe and Canada).ATP-III recommendations were used to classify cardiovascular risk (CVR),LDL-C goal and normal levels of HDL-C and triglycerides (TG).

Results: In 3710 Spanish patients: 69% hypertensive,39% had diabetes mellitus,35.7% had established CVD(23.8% with ischemic heart disease,7.1% with stroke,6.9% with heart failure and 9.3% with peripheral vascular disease).LDL-C was not at goal in 50.4%.The prevalence of low HDL-C was 29.8%,higher in women vs men (35.1% vs 25%,$p< 0.001$), in diabetic patients (28.8% vs 19.5% without diabetes,$p< 0.0001$) and in those with established CVD (31% vs 18.7% without CVD,$p< 0.0001$).The prevalence of CVD was 42.9% in those with low HDL-C and 32.6% in those with normal HDL-C, ($p< 0.0001$).In those with LDL-C not at goal, the prevalence of low HDL-C was 24.0%.The prevalence of low HDL-C was 28.7% in high, 24.8% in moderate and 0.7% in low risk patients.

Conclusions: Prevalence of low HDL-C was frequent in statin treated patients included in this study,especially in high-risk patients (patients with diabetes,CVD or other high risk category).24.0% patients with LDL-C not at goal showed low HDL-C.Low HDL-C could be,in part,responsible for the high remaining CV risk of this population.

Poster Session

ASSOCIATION BETWEEN STRESS-HORMONES AND HEALTH IN MOSCOW POPULATION SAMPLE AGED 55 YEARS AND OLDER

Victoria Metelskaya[1], N. Gumanova[1], A. Deev[1], S. Shalnova[1], M. Shkolnikova[2], V. Shkolnikov[3]

[1]National Research Centre for Preventive Medicine, [2]Federal Center for Cardiac Arrhythmias, Moscow, Russia, [3]Max-Plank Institute for Demography Research, Rostock, Germany

Background: Dysregulation in sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenocortical (HPA) axis activities, mediating cardiovascular effects of stress, contribute to the chronic diseases morbidity and mortality risks.

Aim: To examine the relationship between stress-hormones and health in the high mortality context of Russia.

Methods: Serum DHEA-S and 12-hours urinary cortisol (C) excretion as integrated measure of HPA axis, and urinary epinephrine (E) and norepinephrine (NE) excretion as integrated indices of SNS activity were determined. The sample (n=1876, aged 55+) was drawn from the survey **S**tress **A**nd **H**ealth in **R**ussia (SAHR). Fieldwork occurred in 2006-2009. Relationships between stress-hormones, health outcomes (studied by SF-36, Cohen's Stress score, and MMSE) and conventional cardiovascular risk factors were estimated by regression models controlled by age and sex.

Results: In women, DHEA-S level was 55% lower than in men and declined with age in both sexes. C, E and NE in women were higher by 5.7%, 28% and 25%, respectively, slightly increasing with age. Stress-hormones were associated with BMI, waist circumference, high blood pressure, heart rate, and the following health outcomes: worse physical and mental health (SF-36), mobility limitations, cognitive ability (MMSE), perceived stress (PSS), major life events.

Conclusion: High level of stress-hormones in Russian middle-age and elderly population, characterized by higher mortality compared to other countries, is associated with worth perceived health and higher level of blood pressure. Role of stress for all-cause mortality should be studied further.

The SAHR Study was supported by research grant No R 01 AG 026786 (National Institute of Aging, USA).

Poster Session

PROTECTION AGAINST NEPHROPATHY IN TYPE 2 DIABETES WITH ATORVASTATIN: RELATIONSHIP BETWEEN LIPOPROTEIN PHOSPHOLIPASE A2 AND ADIPONECTIN AND RENAL FUNCTION

Valentine Charlton-Menys, M. Rutter, P. Durrington

University of Manchester, Manchester, UK

Diabetes is the commonest cause of chronic kidney disease in the western world and lipid-lowering therapy could slow its progression. We compared the effects of atorvastatin (10 vs. 80 mg), in 119 patients with type 2 diabetes and early renal disease, on lipoprotein phospholipase A2 and adiponectin levels and assessed their relationship with measures of renal function. Baseline Lp-PLA2 did not differ between groups (464 [365,541] n=59 vs. 472 [412,534] ng/ml, n=60) but was significantly lower at 12 months in both groups (315 [237,405] $p< 0.001$ and 264 [222,328] $p< 0.001$). Using pooled data both serum creatinine (µmol/l) and cystatin C (mg/l) correlated with Lp-PLA2 ($r=0.22$ and $r=0.25$, both $p< 0.05$) at 12 months only, and with adiponectin at baseline ($r=0.26$, $p< 0.005$ and $r=0.30$, $p< 0.005$) and at 12 months ($r=0.25$, $p< 0.05$ and $r=0.30$, $p< 0.005$). Adiponectin also correlated Lp-PLA2 at both visits ($r=0.29$ and $r=0.30$, both $p< 0.005$). Adiponectin correlated inversely with glomerular filtration rate at both visits as assessed using MDRD (-0.24 and $r=-0.25$, both $p< 0.05$) and COGA equations ($r=-0.28$ and $r=-0.41$, both $p< 0.005$). Lp-PLA2 levels >230 ng/ml are associated with high risk of cardiovascular risk and lowering of Lp-PLA2 by atorvastatin may be protective. However, the association of adiponectin with both impaired renal function and Lp-PLA2 may explain why adiponectin does not protect against renovascular disease in diabetes.

Poster Session

ASYMMETRIC DIMETHYLARGININE (ADMA) PREDICTS SKIN BLOOD FLOW DURING HEMODIALYSIS (HD)

Erik Mistrik, V. Blaha, S. Dusilova-Sulkova, R. Safranek, L. Sobotka

Department of Gerontology and Metabolism, University Hospital and Medical Faculty, Charles University, Hradec Kralove, Czech Republic

Background: HD patients suffer from non-healing ischemic skin defects. Elevated plasma levels of ADMA as an endogenous NO synthase inhibitor causes endothelial dysfunction. We hypothesized that inappropriate removal of ADMA during HD causes decrease in skin blood flow.

Methods: Peripheral skin blood flow was measured using Laser Doppler Line Scanner (LDLS®, Moor, Devon, UK) in 10 different areas (AI) of dorsal part of each hand before and during HD (at 1st, 2nd, 3rd and 4th hour) with ultrafiltration in 35 HD patients (14 female, 21 male; age 36-83 y, BMI= 28±5.0). Pre- and post-HD levels of ADMA were measured. Mann Whitney test, Spearman correlation and multivariate regression analysis was used for statistical evaluation.

Results: Post-HD plasma levels of ADMA were elevated in patients with critical skin perfusion (0.46 vs. 0.54 μmol/L; p=0.07). Significant correlations between ADMA and skin blood flow was found on fingers after 1st hour of HD (r=-0.5; p< 0.01) and on dorsum of the hand after 1st (r=-0.44; p< 0.01); 2nd (r=-0.36; p=0.04); 3rd (r=-0.33; p=0.05) and 4th hour of HD (r=-0.36; p=0.04). ADMA was found to be independent predictor of skin blood flow on fingers after 1st hour of hemodialysis.

Conclusion: ADMA predicts skin microcirculation during hemodialysis, which may lead to development of ischemic skin lesions.

Supported by research projects MSM 0021620820, MSM 0021620819, IGA MH CR NR/9743-4.

Poster Session

FAT MASS WAS INVERSELY ASSOCIATED TO ANTIOXIDANT DEFENSE SYSTEM IN OBESE MALE ADOLESCENTS

Ignacio Rosety[1], M.A. Rosety[1], M. Rosety-Rodriguez[2], A. Camacho[3], F.J. Ordonez[1], G. Fornieles[2], M. Rosety[4]

[1]Sport Medicine, [2]Internal Medicine, University of Cadiz, Cadiz, [3]Internal Medicine, Hospital Juan Ramon Jimenez, Huelva, [4]Human Anatomy, University of Cadiz, Cadiz, Spain

Background and aim: It is widely accepted oxidative stress may play a key role in the pathogenesis of atherosclerosis. However little information is available regarding the association of obesity and oxidative damage at early life stages. Consequently, the present study was undertaken to explore whether fat mass and antioxidant defense system are correlated in obese male adolescents.

Methods: Two hundred seventy male adolescents (age 17.2±0.6) volunteered for this study. Two hundred forty of them were obese (BMI=33.8±2.9 kg/m^2; BF=36.2±4.6%) and were randomly selected from. Control group included 30 age and sex-matched adolescents (BMI=22.1±1.8 kg/m^2; BF=25.7±4.2%). No one of them reported neither toxic habits (smoking or alcohol) nor antioxidant consumption. Plasmatic total antioxidant status (TAS) was determined spectrophotometrically by commercial kits supplied by Randox. Fat mass percentage was determined by bioelectrical impedance analysis. Written informed consent was obtained from all their parents. Further our protocol was approved by an institutional ethic committee.

Results: When compared to controls, plasmatic total antioxidant status was lower in obese adolescents (0.62±0.1 vs. 0.44±0.08 mmol/l TAS; $p < 0.05$). A negative but significant association was found between fat mass and total antioxidant status in obese adolescents ($r = -0.52$; $p < 0.05$).

Conclusion: As was hypothesized, fat mass was inversely correlated to total antioxidant status in obese adolescents. Further studies on this topic are highly required to determine whether anthropometric measurements may be considered as easy, economic and non-invasive biomarker of antioxidant defense system at early life stages.

Poster Session

ASSOCIATION OF VARIOUS POLYMORPHISMS WITH CAD IN NORTH INDIAN POPULATION: REPLICATION OF SNPS IDENTIFIED THROUGH GENOME WIDE ASSOCIATION STUDIES

Jitender Kumar[1,2], S. Yumnam[1], T. Basu[1], A. Ghosh[1], G. Garg[1], G. Karthikeyan[3], S. Sengupta[1]

[1]Institute of Genomics and Integrative Biology, Delhi, India, [2]Department of Osteoporosis, Lund University, Malmö, Sweden, [3]All India Institute of Medical Sciences, New Delhi, India

Introduction: Several Genome Wide Association Studies (GWAS) have identified novel single nucleotide polymorphisms (SNPs) that show association with coronary artery disease (CAD) and myocardial infarction. We analyzed SNPs identified through these GWAS and tried to establish their association with CAD status in North Indian population.

Materials and methods: A total of 739 individuals (296 angiography confirmed CAD patients and 443 treadmill test controls) were recruited from a tertiary care center situated in New Delhi, India. A total of six different SNPs (five from locus 9p21 i.e. rs10116277, rs10757274, rs1333040, rs2383206, rs2383207 and one from chromosome 15 i.e. rs1994016) identified through GWA studies (The Wellcome Trust Case Control Consortium, 2007; McPherson et al., 2007 and Helgadottir et al., 2007) were selected and their association with the CAD status was analyzed. Logistic regression analysis was performed to check the association after controlling for various confounding factors.

Results: The minor allele frequency (MAF) observed for the six SNPs was comparable with the original GWA studies. All the SNPs conformed to Hardy-Weinberg equilibrium. In the logistic regression analysis, five SNPs present at the locus 9p21 were found to be significantly associated with CAD status after controlling for confounding factors including age, sex, BMI, diet, hypertension, diabetes, smoking and levels of homocysteine, vitamin B12 and folate (p value=0.05-0.006).

Conclusion: SNPs present at locus 9p21 that are identified through GWAS conducted in the Caucasian populations and associated with cardiovascular diseases are also important in North Indian population.

Poster Session

PLANT AND ANIMAL PROTEIN CONSUMPTION ARE ASSOCIATED WITH LIKELIHOOD OF DEVELOPING LEFT VENTRICULAR SYSTOLIC DYSFUNCTION, IN ACUTE CORONARY SYNDROME PATIENTS

Christina-Maria Kastorini[1], C. Chrysohoou[2], D.B. Panagiotakos[1], P. Aggelopoulos[2], C. Pitsavos[2], C. Stefanadis[2]

[1]Department of Nutrition Science - Dietetics, Harokopio University, [2]First Cardiology Clinic, School of Medicine, University of Athens, Athens, Greece

Background & aims: The aim of the present work was to evaluate the association between plant and animal protein consumption and the development of left ventricular systolic dysfunction (LVSD) in patients who have had an acute coronary syndrome (ACS).

Methods: During 2006-2009, 1000 consecutive ACS patients were included in the study; 459 patients who developed LVSD, 367 males (64±14 years) and 92 females (71±12 years) and 541 patients with preserved systolic function, 421 males (62±12 years) and 120 females (67±12 years). Detailed information regarding their medical records, anthropometric data, physical activity and smoking habits were recorded. Nutritional habits were assessed using a semi-quantitative food-frequency questionnaire and macronutrient consumption was evaluated.

Results: Multi-adjusted analysis after adjustment for various confounding factors revealed that in patients with first coronary event, plant protein intake at the third quartile of consumption was associated with 60% (95%CI:0.17-0.95) lower likelihood of developing LVSD, compared to the first. Furthermore in patients with previous coronary heart disease history, animal protein intake at the highest quartile of consumption, was associated with 211% greater likelihood of developing LVSD (95%CI:1.08-8.98), compared to the first.

Conclusions: Plant protein consumption seems to be beneficial, while animal protein consumption detrimental, against the development of LVSD in post-ACS patients.

Poster Session

MODERATE COFFEE CONSUMPTION LOWERS THE LIKELIHOOD OF DEVELOPING LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN POST-ACUTE CORONARY SYNDROME NORMOTENSIVE PATIENTS

Christina-Maria Kastorini[1], C. Chrysohoou[2], D.B. Panagiotakos[1], P. Aggelopoulos[2], C. Liontou[2], C. Pitsavos[2], C. Stefanadis[2]

[1]Department of Nutrition Science - Dietetics, Harokopio University, [2]First Cardiology Clinic, School of Medicine, University of Athens, Athens, Greece

Background & aims: The aim of the present work was to evaluate the association between coffee consumption and the development of left ventricular systolic dysfunction (LVSD) in patients who had had an acute coronary syndrome.

Methods: During 2006-2007, 144 male (65±14 years) and 50 female (71±12 years) post-acute coronary syndrome patients who developed LVSD (ejection fraction < 40%) after the cardiac event and 129 male (64±12 years) and 51 female (67±10 years) post-acute coronary syndrome patients without LVSD (ejection fraction >50%) were included in the study. Detailed information regarding their medical records, sociodemographic and anthropometric data, and various psychological and lifestyle characteristics (physical activity, smoking habits, etc.) were recorded. In particular, nutritional habits, including coffee consumption, were evaluated using a semiquantitative food-frequency questionnaire.

Results: Multiadjusted analysis revealed that in normotensive patients coffee consumption of 1-2 cups/day was associated with 88% (95% confidence interval, 0.02-0.84) lower likelihood of developing LVSD and consumption of >3 cups/day with 90% (95% confidence interval, 0.01-0.88) lower likelihood for LVSD, compared with no history of consumption of coffee and after adjusting for various confounders. In contrast, in hypertensive patients coffee consumption of >3 cups/day was associated with 4.5-fold higher likelihood for developing LVSD (95% confidence interval, 0.89-22.58) as compared with no history of coffee consumption.

Conclusions: Coffee consumption has opposite effects on the likelihood of developing LVSD in post-acute coronary syndrome patients depending on their blood pressure levels.

Poster Session

COMPARATIVE MAPPING OF LINKAGE AND ASSOCIATION SIGNALS IN MYOCARDIAL INFARCTION

Anja Medack[1], C. Loley[2], J. Nahrstaedt[2], I.R. König[2], B. Mayer[1], C. Hengstenberg[3], K. Stark[3], H.-E. Wichmann[4], S. Schreiber[5], T. Meitinger[4], H. Schunkert[1], J. Erdmann[1]

[1]Medizinische Klinik II, [2]Institut für Medizinische Biometrie und Statistik, University of Luebeck, Luebeck, [3]Klinik und Poliklinik für Innere Medizin II, University Regensburg, Regensburg, [4]Institut für Epidemiologie, Helmholtz Zentrum, Neuherberg, [5]Institut für Klinische Molekularbiologie, University of Kiel, Kiel, Germany

Our group established a large collection of myocardial infarction (MI)-families with 2-5 affected first-degree relatives and large collection of unrelated MI cases and controls. Genome-wide microsatellite linkage analyses on MI sibling-pairs and three genome-wide association (GWA) studies on unrelated MI cases (n=875 [GerMIFSI]/ n=1222 [GerMIFSII]/ n=1157 [GerMIFSIII]) and controls (n=4690) were carried out. Our current approach assumes that the same genes involved in rare autosomal-dominant forms of MI may also be associated with the risk for MI in the general population.

25 families with an autosomal-dominant inheritance pattern were analysed by cosegregation analyses using microsatellite markers. Genome-wide scanning was performed using high-density SNP arrays. Data analysis was performed in a three-step procedure:

1. Comparison of linkage and association data

2. Replication in the GWAS

3. Sequencing of these regions.

With the genome-wide microsatellite linkage analysis in 25 families (n=559 individuals) five chromosomal regions 1q42.3; 4q31.21: 4q34.1; 8q24.13 and 17q24.2 were identified for suggestive linkage. In the same chromosomal regions we found association for several SNPs tested in three GWAS. With data analyses we reduced the chromosomal region to top regions encompassing only few genes or intergenic regions. For example, the chromosome 4 locus (~320 genes) was reduced to four top regions, including *MND1* and *GALNT17*.

The comparative analysis of linkage and GWAS data in families and case/control samples with MI allows the condensation of linkage intervals leading to a much smaller number of genes to be sequenced in order to identify the underlying mutations in familial forms of MI.

Poster Session

ASSIMILATED ANALYSIS OF FIRST WAVE HITS FROM GENOME WIDE ASSOCIATION STUDIES WITH BLOOD BIOMARKERS AND CORONARY EVENTS

Aspasia Angelakopoulou, on behalf of the Cardiovascular Biomarker Genetics Collaboration

London School of Hygiene and Tropical Medicine, London, UK

Objective: To conduct an integrated association analysis of the effects of 34 GWAS hits of blood lipids, adiposity, T2DM and CHD on a wider range of cardiovascular biomarkers and CHD risk.

Methods: We used information from up to 30,000 individuals from two case-control, three cross-sectional and seven prospective studies, including up to 3,872 CHD and 2,340 T2DM cases. We used random effect meta-analysis to pool study-specific estimates supplemented where relevant with data from the Wellcome Trust Case-Control Consortium 1.

Results: Of the 14 SNPs originally identified with CHD risk, only the two chromosome 9p21 rs1333049 (per-allele OR 1.25; 95% CI, 1.05-1.50); and rs10757274 (per-allele OR 1.15; 95% CI, 1.02-1.29), were replicated in this data set. These SNPs, however, showed no association with any of the 16 CHD biomarkers measured. Of the 20 SNPs identified through an association with either triglycerides, HDL-C, or LDL-C, only rs17411031 in *LPL* showed a protective CHD effect (per-allele OR 0.89; 95% CI, 0.81-0.97), a higher level of APOA1 and HDL-C as well as lower level of triglycerides, but showed no association with LDL-C.

Conclusions: The risk of CHD conferred by carriage of chromosome 9p21 SNPs is unlikely to be mediated through blood pressure, lipids, inflammation biomarkers or glycaemic control. The association of rs17411031 with traits other than LDL-C suggests target mechanisms other than LDL-lowering one may be helpful for CHD prevention. Systematic, comprehensive and large-scale genetic and phenotypic information should enable a deeper understanding of genetic-disease mechanisms and the identification of potential drug targets.

Poster Session

UNIVARIATE ANTHROPOMETRIC MEASURES FOR PREDICTING MAJOR CARDIOVSCULAR EVENTS MAY BE MISLEADING

Andreas Rosenblad, G. Nilsson, J. Leppert

Center for Clinical Research Västerås, Uppsala University, Västerås, Sweden

Purpose: To compare the univariate and multivariate predictive ability for Major Cardiovascular Events (MCVE) of the anthropometric measures Body Mass Index (BMI), Waist Circumference (WC), Waist-Hip Ratio (WHR), Waist-Height Ratio (WHtR) and Waist-Hip-Height Ratio (WHHR).

Methods: All men and women aged 40 and 50 from the county of Västmanland, Sweden (260000 inhabitants) were invited to a health examination during 1990-99 (participation rate=48%). MCVE, defined as death or hospitalization with ICD-10 codes I21-I22, I62-I66 or I70-I74, were followed up until December 31, 2006. For persons with complete anthropometric measures (n=33493, men=48%), Cox regression was performed for BMI, WC, WHR, WHtR and WHHR (defined as WHR divided by height). Multivariate adjustment added age, diabetes, smoking, systolic and diastolic blood pressure, heart rate and glucose level. P-values of Hazard Ratios (HR) and predictive abilities (calculated using a Pseudo R^2 measure) of each measure were computed.

Results: During follow-up 1212 persons (3.6%, men=67%) experienced MCVE. All HR p-values were significant at $p< 0.05$. The univariate predictive abilities were high for all measures: BMI (men=0.067, women=0.122), WC (0.083, 0.194), WHR (0.115, 0.226), WHtR (0.116, 0.208), WHHR (0.138, 0.222). However, after adjusting for confounders the increase in predictive ability when adding anthropometric measures was small: BMI (0.006, 0.004), WC (0.007, 0.011), WHR (0.010, 0.016), WHtR (0.011, 0.012), WHHR (0.013, 0.014).

Conclusions: It may be misleading to rely on univariate statistical methods for estimating the influence of anthropometric measures on MCVE for nonrandomised studies. Multivariate methods should be the main statistical method for this kind of studies.

Poster Session

THE CONNECTIONS OF *ADAM8*S (A DISINTEGRIN AND METALLOPROTEASE) GENETIC POLYMORPHISMS TO CORONARY ARTERY DISEASE AND MYOCARDIAL INFARCTION

Emma Raitoharju[1], M. Levula[1], P. Kuukasjärvi[2], J. Laurikka[2], K. Nikus[3], A.-P.J. Huovila[4], R. Laaksonen[1], P.J. Karhunen[5], J. Viik[6], M. Tarkka[2], M. Kähönen[7], T. Lehtimäki[1]

[1]Department of Clinical Chemistry, [2]Heart Center, Department of Cardiothoracic Surgery, [3]Department of Cardiology, Tampere University Hospital and Tampere University Medical School, [4]Institute of Medical Technology, University of Tampere, [5]Department of Forensic Medicine, Tampere University Hospital and Tampere University Medical School, [6]Department of Biomedical Engineering, Tampere University of Technology, [7]Department of Clinical Physiology, Tampere University Hospital and Tampere University Medical School, Tampere, Finland

Background: In earlier studies we have shown that ADAM8 (a disintegrin and metalloproteinase 8) is highly expressed in complicated atherosclerotic lesions and the C-allele carriers of ADAM8 rs2995300 (A/C) (ADAM8-2662) SNP locus have higher advanced atherosclerotic lesion areas in their coronary arteries and a significantly higher risk of fatal myocardial infarctions than non-carriers.

Aims: The aims of this study were to replicate our earlier findings in two independent clinical cohorts. In addition, we investigate whether ADAM8 rs2275725 (A/G) (ADAM-35) polymorphisms, which is inherited together with ADAM8-2662 or their haplotypes affects to the risk of myocardial infarction.

Materials and methods: Two independent cohorts consisting more than 3000 subjects participating to The Finnish Cardiovascular study (FINCAVAS, n=2156) and Angiography and Genes (ANGES, n=1000) study was used as study populations in present study. The genotypes were investigated by TaqMan based 5'-exonuclease assay and fluorogenic allele specific probes. Statistical analyses were done by SPSS, PHASE and LDA programs.

Results: In logistic regression analyses both ADAM8-2662 SNP C-allele carriers and ADAM8-35 A-allele carriers have significantly higher risk for MI in both used cohorts as compared to patient without these alleles (ANGES: OR=1.6 CI 95% 1.16-2.21, p=0.005 and OR=1.60 CI 95% 1.14-2.24, p=0.007, respectively and FINCAVAS: OR=1.32 CI 95% 1.03-1.69, p=0.026 and OR=1.35 CI 95% 1.04-1.74, p=0.02, respectively). The major results remain significant after adjusting by conventional risk factors.

Conclusions: The investigated ADAM8 polymorphisms raise significantly the risk of MI corresponding the level of smoking in the two independent Finnish population cohorts.

Poster Session

ADMA (ASYMMETRIC DIMETHYLARGININE) PREDICTS ALL-CAUSE MORTALITY AND CARDIOVASCULAR EVENTS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE: THE GETABI STUDY

Maike Anderssohn[1], R.H. Böger[1], H.G. Endres[2], E. Schwedhelm[1], H. Darius[3], D. Atzler[1], N. Lüneburg[1], B. von Stritzky[4], R. Maas[5], U. Thiem[6], R.A. Benndorf[7], C. Diehm[8]

[1]Institute of Experimental and Clinical Pharmacology and Toxicology, Universitätsklinikum Hamburg-Eppendorf, Hamburg, [2]Department of Medical Informatics, Biometry and Epidemiology, Ruhr University Bochum, Bochum, [3]Department of Medicine I, Vivantes Berlin-Neuköllln Medical Center, Berlin, [4]Medical Department, Sanofi-Aventis Pharma GmbH, Berlin, [5]Institute of Clinical Pharmacology and Toxicology, University of Erlangen, Erlangen, [6]Department of Geriatrics, Marienhospital Herne, Ruhr University Bochum, Herne, [7]Institute of Pharmacology, Toxicology and Clinical Pharmacy, Technical University Braunschweig, Braunschweig, [8]Department of Internal Medicine / Vascular Medicine, Klinikum Karlsbad-Langensteinbach, Karlsbad, Germany

Background: Peripheral arterial disease (PAD) confers a high risk of vascular events and mortality. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis associated with endothelial dysfunction, whereas SDMA does not inhibit NO synthases.

Methods and results: We investigated the association of plasma ADMA and SDMA with the prevalence of PAD and the incidence of major cardiovascular events and death in the multicenter getABI trial. From a cohort of 6,821 primary care patients, 1,260 patients with prevalent PAD were compared to a random sample of 1,187 non-PAD patients. ADMA and SDMA were measured by a validated LC-MS/MS method. Mean plasma ADMA concentration was slightly, but significantly, higher in PAD patients than in controls (0.63±0.14 µmol/l vs. 0.61±0.13 µmol/l; p< 0.001). Mean SDMA concentration was 0.52±0.16 µmol/l in PAD patients and 0.48±0.13 µmol/l in controls (p< 0.001). For SDMA there was a highly significant interference with eGFR (p< 0.001). ADMA and SDMA levels were not significantly different between patients with symptomatic or asymptomatic PAD. During prospective follow-up, ADMA levels above the 3rd quartile were associated in a multiple adjusted Cox regression with a hazard ratio of 1.41 (1.14-1.74; p = 0.002) for total mortality, 1.32 (1.03-1.69; p = 0.028) for severe cardiovascular disease events, and 1.50 (0.98-2.29; p = 0.061) for severe cerebrovascular disease events.

Conclusion: ADMA and SDMA are independent markers of mortality risk in ambulatory patients with PAD. The association of SDMA and mortality is in part explained by a close link to renal function.

Poster Session

CARDIOVASCULAR RISK SCORES IN THE PREDICTION OF SUBCLINICAL ATHEROSCLEROSIS IN YOUNG ADULTS

J. Raiko[1], **Costan Magnussen**[1], M. Kivimäki[2], L. Taittonen[3], T. Laitinen[4], M. Kähönen[5], N. Hutri-Kähönen[6], A. Jula[7], B.-M. Loo[7], R. Thomson[8], T. Lehtimäki[9], J. Viikari[10], O. Raitakari[11], M. Juonala[10]

[1]Research Center of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, [2]Finnish Institute of Occupational Health, Finnish Institute of Occupational Health, Helsinki, [3]Department of Pediatrics, University of Oulu, Oulu, [4]Department of Clinical Physiology, Kuopio University Hospital, Kuopio, [5]Department of Clinical Physiology, [6]Department of Pediatrics, University of Tampere and Tampere University Hospital, Tampere, [7]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Turku, Finland, [8]Group of Biostatistics, Menzies Research Institute, Hobart, TAS, Australia, [9]Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, [10]Department of Medicine, [11]Department of Clinical Physiology, University of Turku, Turku, Finland

Aims: To study the utility of risk scores in prediction of subclinical atherosclerosis in young adults.

Methods: Participants were 2,204 healthy Finnish adults aged 24-39 years in 2001 (1,210 females; 994 males) from population-based follow-up study Cardiovascular Risk in Young Finns. We examined the performance of the Framingham, Reynolds, SCORE (Systematic Coronary Risk Evaluation), PROCAM, and Finrisk cardiovascular risk scores to predict non-invasive ultrasound measurements of carotid artery intima-media thickness and plaque (IMT), carotid artery distensibility (CDist) and brachial artery flow-mediated dilatation (FMD) at 6 years. High IMT was defined as highest decile in IMT and/or carotid plaque and low Cdist and low FMD as lowest deciles in Cdist and FMD.

Results: In 6-year prediction of high IMT, areas under the receiver operating characteristic curves (AUC) for baseline Finrisk (0.733), SCORE (0.726), PROCAM (0.712) and Reynolds (0.729) risk scores were similar as for Framingham risk score (0.728, P always ≥0.15). All risk scores had similar discrimination in predicting low CDist (0.652, 0.642, 0.639, 0.658, 0.652 respectively, P always ≥0.41). In prediction of low FMD, Finrisk, PROCAM, Reynolds and Framingham scores had similar AUCs (0.578, 0.594, 0.582, 0.568, P always ≥0.08) and SCORE discriminated slightly better (AUC=0.596, P< 0.05). Prediction of subclinical outcomes was consistent when estimated from other statistical measures of discrimination, reclassification, and calibration.

Conclusions: CVD risk scores had equal performance in predicting subclinical atherosclerosis measured by IMT and CDist in young adults. SCORE was more accurate at predicting low FMD than Framingham risk score.

Poster Session

IMPACT OF CARDIOVASCULAR MULTI-MORBIDITY ON PERSISTENT DYSLIPIDEMIA IN PATIENTS TREATED WITH STATINS IN GERMANY: LESSONS LEARNED FROM DYSIS

Wenefrieda Smolka[1], C. Juenger[2], K. Bestehorn[1], A.K. Gitt[2]

[1]MSD Sharp & Dohme GmbH Haar, Haar, [2]Institut fuer Herzinfarktforschung Ludwigshafen an der Universitaet Heidelberg, Ludwigshafen, Germany

Aims: Most patients with dyslipidemia in Germany are treated with statins but many do not achieve lipid goals/normal levels; Persistent lipid abnormalities continue to contribute to cardiovascular risk and morbidity in these patients. We evaluated the impact of cardiovascular multi-morbidity (CV-MM) on the lipid profile in patients already on a statin treatment.

Methods: DYSIS (Dyslipidemia International Study) was an epidemiological cross-sectional study to assess the lipid profile as well as the patients´ characteristics of 22,063 consecutive statin-treated patients during a single visit to their physicians (2987 centres) on an outpatient basis in Europe and Canada. A total of 4244 patients were enrolled in 748 centres in Germany. CV-MM was defined as prevalence of 3-6 cardiovascular diseases or risk factors including hypertension, diabetes mellitus, ischemic heart disease, cerebrovascular disease, heart failure, peripheral artery disease.

Results: Patients with CV-MM were more likely to reach LDL-C target values under statin treatment in clinical practice (49% not at goal) as compared to patients without CV-MM (63%;$p< 0.0001$), but at the same time had a higher prevalence of low HDL-C (29 vs. 19%;$p< 0.0001$) and elevated triglycerides (51 vs. 45%;$p< 0.01$).

Conclusion: Despite chronic statin treatment, half of patients with CV-MM were not at goal for LDL-C in clinical practice. One third had low HDL-C and 50% elevated triglycerides. Results demonstrate a gap between guideline recommendations and clinical practice and the need for a more comprehensive lipid management addressing not only LDL-C but also HDL-C and triglycerides in the high risk population with CV-MM.

Poster Session

PRE-CLINICAL EFFICACY OF DRL-17822: A POTENT ORALLY ACTIVE CETP INHIBITOR

Alikunju Shanavas, K. Ramesh, S. Akhila, K. Ranjan, K. Rajesh, S.N. Hasham, B. Anima, I. Khanna, R. Pillarisetti, R. Kumar

Dr. Reddy's Laboratories Ltd., Hyderabad, India

Introduction: Epidemiologic studies have shown an inverse correlation between HDL-C levels and the risk of atherosclerotic cardiovascular disease. Effective therapies to increase plasma HDL-C represent an area of unmet medical need. Inhibition of CETP is an emerging strategy for elevating HDL-C levels, with human proof of concept being established in clinical trials.

Objectives: The present study evaluates the HDL-C elevating potential of DRL-17822, a novel and potent inhibitor of human CETP (IC_{50}- 18 ± 7 nM) in hyperlipidemic hamsters and rabbits. Haemodynamic effects were evaluated using a rat model.

Methods: DRL-17822 was administered orally to hamsters (0.19 to 12 mg/kg), rabbits (24 mg/kg) and rats (25 and 100 mg/kg) for 7, 14 and 21 days respectively. At the end of treatment period plasma lipid parameters were assayed in hamsters & rabbits and effects on blood pressure and heart rate were measured in rats.

Results: DRL-17822 dose dependently elevated HDL-C in hamsters by 8, 24, 29, 39, 50, 73 and 65% at 0.19, 0.38, 0.75, 1.5, 3, 6 and 12 mg/kg respectively (ED_{50}-1.2 mg/kg, $p< 0.05$). Robust elevation of HDL-C was also observed in rabbits treated with DRL-17822 (251% at 24 mg/kg, $p< 0.05$). DRL-17822 did not cause any significant changes in blood pressure and heart rate whereas torcetrapib showed significant elevation in systolic, diastolic and mean arterial pressure in rats.

Conclusions: These results demonstrate that DRL-17822 significantly elevates HDL-C in pre-clinical animal models without any inherent cardiovascular liabilities including no significant effect on blood pressure.

Poster Session

CORONARY PATIENTS REACHING LDL-CHOLESTEROL < 2.0 MMOL/L HAVE THE LOWEST RISK OF CARDIOVASCULAR EVENTS DURING STATIN TREATMENT: THE IDEAL STUDY

Anders Olsson[1,2], C. Lindahl[3], I. Holme[4], R. Fayyad[5], O. Faergeman[6], J. Kastelein[7], M. Tikkanen[8], M. Lytken Larsen[6], T. Pedersen[4], on behalf of the Incremental Decrease in End Points through Aggressive Lipid Lowering (IDEAL) Study Group

[1]Department of Internal Medicine, University Hospital, Linkoping, [2]Stockholm Heart Center, Stockholm, [3]Pfizer Sweden, Sollentuna, Sweden, [4]Center of Preventive Medicine, Oslo University Hospital, Oslo, Norway, [5]Pfizer Inc, New York, NY, USA, [6]Department of Medicine-Cardiology, Arhus University Hospital, Arhus, Denmark, [7]Academic Hospital Amsterdam, Amsterdam, The Netherlands, [8]Helsinki University Hospital, Helsinki, Finland

Object: We assessed how patients treated with either 20 to 40 mg of simvastatin or 80 mg of atorvastatin were able to achieve LDL cholesterol (LDL-C) goals of 2.5 or 2.0 mmol/L in the IDEAL study and explored to what extent those reaching the 2.0 mmol/L goal fared better clinically than those reaching the 2.0-2.5 mmol/L levels and how lipoprotein components related to cardiovascular disease (CVD) outcome in these groups.

Methods and results: For subjects who reached on-study LDL-C goals, Cox regression models were used to assess the ability of on-treatment apolipoprotein B (apoB), apoB/apoA1 and non-HDL-C to predict CVD. More CVD patients treated with atorvastatin than simvastatin were able to reach the LDL-C goals of 2.5 and 2.0 mmol/L. With adjustments for baseline LDL-C levels, hazard ratios for all participants between those reaching 2.0 mmol/L LDL-C versus those reaching 2.0-2.5 mmol/L range were 1.16 (95% confidence limits: 1.02-1.33; p=0.023). Increase of the lipoprotein components apoB/A1 ratio by 1 standard deviation in participants who reached 2.0 mmol/L showed a hazard ratio for CVD of 1.14 (1.04-1.25, p=0.004).

Conclusion: Those reaching the LDL-C goal of 2.0 mmol/L exhibit significantly less CVD than those reaching only 2.0-2.5 mmol/L. In those reaching the LDL-C goal of 2.0 mmol/L the apoB/A1 ratio still bears a relation to CVD outcome. Therefore in CVD patients the LDL-C goal should be 2.0 mmol/L. The use of apoB/A1 in monitoring CVD patients on statin treatment is superior to LDL-C.

Poster Session

PEDIATRIC METABOLIC SYNDROME IN PREDICTING METABOLIC SYNDROME, HIGH CAROTID INTIMA-MEDIA THICKNESS, AND TYPE II DIABETES MELLITUS IN ADULTHOOD

Costan G. Magnussen[1], J. Koskinen[1], M. Juonala[1], S.R. Srinivasan[2], J.S.A. Viikari[1], G.S. Berenson[2], O.T. Raitakari[1]

[1]University of Turku and Turku University Hospital, Turku, Finland, [2]Tulane University, New Orleans, LA, USA

Background: The clinical utility of identifying pediatric metabolic syndrome (MetS) is controversial. This study determined: the long-term (childhood to adulthood) stability of MetS; and if those with pediatric MetS are at increased risk of MetS, high carotid intima-media thickness (IMT), and type 2 diabetes mellitus (T2DM) in adulthood.

Methods: Using data from the population-based prospective Bogalusa Heart and Cardiovascular Risk in Young Finns studies, we examined the utility of four categorical definitions of youth MetS to predict MetS, high IMT, and T2DM amongst 1781 adults after 24-years follow-up.

Results: We found that approximately 60%, 80%, and 95% of youth identified as having MetS would not have MetS, high IMT, or T2DM with most adults with MetS acquiring it in the time between youth and adulthood. Irrespective of poor stability, youth with MetS were at 2-3 times the risk of having MetS, high IMT, and T2DM as adults compared with those that did not have MetS as youth. Nevertheless, comparisons of area under the receiver operating characteristic curve and reclassification data suggested that prediction of MetS, high IMT, and T2DM in adulthood using youth MetS was either equivalent or significantly reduced when compared with high body mass index or being overweight or obese in youth.

Conclusions: Although youth MetS predicts important outcomes in adulthood, the identification of youth who are overweight or obese might provide a more cost-effective and clinically useful approach to determining those at increased risk of MetS, high IMT, and T2DM in adulthood.

Poster Session

ESTIMATION OF OXIDATIVE STRESS MARKERS AND THEIR CONNECTION WITH ATHEROSCLEROSIS RISK FACTORS IN CHRONIC KIDNEY DISEASE PATIENTS

Agnieszka Stencel[1], A. Pacanis[1], B. Kortas-Stempak[1], A. Ćwiklińska[1], M. Wróblewska[2], B. Rutkowski[3], M. Ziętkiewicz[3], M. Renke[3]

[1]Department of Clinical Chemistry, [2]Department of Laboratory Medicine, [3]Chair and Department of Nephrology, Transplantology and Internal Diseases, Medical University, Gdansk, Poland

Changes mediated by oxidative stress are thought to be involved with atherosclerosis in patients with chronic kidney disease (CKD). The aim of this study was to analyse lipids and proteins oxidative damage markers in patients with different stage of CKD, both dialysed and non dialysed. The following groups were enrolled in the study: patients with CKD under conservative treatment (CT), peritoneal dialysis patients (PD), haemodialysis patients (HD) and healthy persons as the control group. We measured serum malonodialdehyde (MDA) and ox-LDL as the markers of lipids peroxidation and advanced oxidative protein products (AOPP), as the trace of proteins modification. Additionally serum creatinine, albumin, CRP, lipids profile, apo-B and apo-A-I were measured in each patient. Our results (Table 1) show that uremic patients had higher levels of all studied plasma oxidative stress markers compared with the control group. We conclude also that AOPP, which increased gradually in the following stages of CKD and correlated significantly with HDL ($r=-0,4$ $p< 0,001$) and CRP ($r=0,3$ $p< 0,005$) seems to be especially important by estimation oxidative damage in patients with CKD and may be potential target to interrupt the vicious circle of kidney disease and cardiovascular complications.

	Control N=31	CT III stadium N=7	CT IV stadium N=16	CT V stadium N=18	PD N=47	HD N=38
MDA [µmol/L]	3,2±0,5	3,9±0,5*	3,8±0,6*	4,5±0,6*	4,6±0,8*	4,2±0,7*
ox-LDL [U/L]	65±22	86±8*	90±10*	85±15*	89±23*	79±24*
AOPP [µmol/L]	88±27	112±24*	216±81*	254±97*	146±38*	187±66*

[Tabele 1 Markers of oxidative modification]
The values are presented as the mean±SD, *significant differences between patients and control ($p< 0,05$).

Poster Session

ELEVATED TRIGLYCERIDE / HDL CHOLESTEROL RATIO IS A PREDICTOR OF POOR RENAL FUNCTION IN A LARGE TERTIARY REFERRAL LIPID CLINIC

Joseph Davies[1], D. Goldsmith[2,3], M. Crook[2], A. Wierzbicki[2]

[1]Medical Student, Kings College London, [2]Lipid Department, [3]Renal Medicine, Guy's and St Thomas' Hospitals, London, UK

Introduction: The prevalence in the general population of chronic kidney disease (CKD) - stage 3-5 (GFR < 60 mls/min) is 4.7% and 11% for proteinuria (ACR>2.4 mg/mmol or urinary protein>0.06 g/l). The role dyslipidemia may play in the development of CKD is unclear. As CKD is so closely linked with CVD, we wanted to examine the impact of the triglyceride / HDL cholesterol ratio (tg:HDL-C)- a predictor of the insulin resistant state and CVD- on kidney function and proteinuria.

Methods: 1283 patients came in 2008, of which 1029 had complete serum lipid and renal profiles. Estimated GFR was calculated for these patients using the 4 variable MDRD formula. We investigated the correlation between the tg:HDL-C and CKD / proteinuria prevalence using 1.55 as the normal boundary. 1.55 was derived from the national cholesterol education program guidelines.

Results: The prevalence of CKD and proteinuria in 1029 patients was 11.18% and 19.42% respectively. In individuals with a tg:HDL-C ratio< 1.55 (n=653) the prevalence of CKD was 9.04%, increasing to 14.89% in those with a tg:HDL-C ratio≥1.55 (n=376), (RR 1.56, 95% CI 1.11 to 2.21, p=0.0041). A high (≥1.55) tg:HDL-C ratio was also associated with an increased incidence of proteinuria: 15.01% vs. 27.13% (RR 1.63, 95% CI 1.27 to 2.10, p< 0.0001).

Conclusion: Using a large patient database of 1,029 patients from a tertiary referral lipid clinic it is clear that both CKD and proteinuria are both common. In this population there is a marked effect on the prevalence of renal dysfunction when the tg:HDL-C ratio is raised.

Poster Session

HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 INFECTION AS A RISK FACTOR FOR ATHEROSCLESOSIS

Hiroaki Takeoka[1], N. Furusyo[1], K. Toyoda[1], M. Murata[1], H. Ohnishi[1], S. Maeda[1], Y. Sawayama[1], Y. Sagara[2], S. Kashiwagi[2], J. Hayashi[1]

[1]Department of General Internal Medicine, Kyushu University Hospital, [2]Japanese Red Cross Fukuoka Blood Center, Fukuoka, Japan

The infection was known as a causative agent of atherosclerosis by the induction of inflammatory components. However, the effect of human T-lymphotropic virus type 1 (HTLV-1) infection on atherosclerosis has not been investigated yet. To clarify the influence of HTLV-1 on atherosclerosis, we examined parameters for atherosclerosis in the carriers.

In 1478 under health examination in Iki island, one of the endemic area of HTLV-1, 272 showed positive for anti-HTLV-1 antibody in particle agglutination assay and were diagnosed as HTLV-1 carriers. The maximum carotid intima-media thickness (MAX-IMT) and brachial-ankle pulse wave velocity (baPWV) were measured as atherosclerosis parameters.

Interestingly, HTLV-1 carriers showed significantly higher values of both of MAX-IMT and baPWV than those in (negative) control. Moreover, in multiple regression analysis for MAX-IMT over 1.1mm, HTLV-1 infection was found as an independent factor for development of atherosclerosis. Serum monocyte chemoattractant protein-1 increased only in carriers along with progression of atherosclerosis. Especially in youth (under 50 years old), Max-IMT of HTLV-1 carriers was significantly higher (0.90±0.08) than that of control (0.74±0.01).

This is the first report that HTLV-1 infection caused early onset of atherosclerosis, indicating that HTLV-1 infection is one of risk factors for atherosclerosis.

Poster Session

GENETIC SCREENING AND ASSOCIATION STUDY OF SERUM AMYLOID A1 (SAA1), AN ACUTE PHASE PROTEIN WITH A POSSIBLE ROLE IN ATHEROSCLEROSIS

Koon Yeow Leow

Department of Paediatrics, National University of Singapore, Singapore, Singapore

Acute-phase SAA (A-SAA) is an acute phase, apolipoprotein that is upregulated during an acute-phase reaction. A-SAA is reported to be an inducer of cytokine secretion from monocytes and macrophages as well as a facilitator of cholesterol efflux from macrophages. The aim of this study is to identify genetic variants of SAA1 in a Singaporean Chinese population and to perform genetic association study to ascertain whether these variants are associated with increased susceptibility for CAD. Genetic screening was performed using high resolution amplicon melting analysis. A total of seven single nucleotide polymorphism (SNP) were identified in the exon and promoter regions of which three were novel. Two novel SNPs, *IVS2+17T>G* and *IVS2-5T>G*, were identified in the exon-intron boundaries and a novel SNP,*-250G>A*, was discovered in the promoter region. Genetic association study of *IVS2+17T>G, IVS2-5T>G, -250G>A* and a previously reported rare variant, *215G>A (72G>D)*, with CAD was performed in the Singaporean Chinese population. There was no significant association between *IVS2+17T>G, IVS2-5T>G* and *-250G>A* with CAD susceptibility. However, the *A* allele of 215G>A was significantly more frequent in the CAD patients (0.014) compared to the control group (0.005; $P< 0.05$). In a functional study performed using human THP1 macrophages, the wild-type recombinant SAA1 has two fold higher induction of interleukin 8 compared to the *215A* allele. Therefore, we concluded that SAA1 might play a role in the development of CAD and that this might be mediated by a mutation in the protein that alters its functional role.

Poster Session

CHRONIC KIDNEY DISEASE IS COMMON IN THE SETTING OF A LARGE TERTIARY REFERRAL LIPID CLINIC

Joseph Davies[1], D. Goldsmith[2,3], M. Crook[2], A. Wierzbicki[2]

[1]Medical Student, King's College London, [2]Lipid Department, [3]Renal Medicine, Guy's and St Thomas' Hospitals, London, UK

Introduction: The prevalence of chronic kidney disease (CKD), defined as eGFR < 60 ml/min/1.73 m^2 for greater than 3 months, in the general population is 4.7%. Hypertension is a well established risk factor for the development of CKD, however recently it has been proposed that dyslipidemia may also contribute. We investigated the incidence of CKD in familial hypercholesterolemic (FH) patients attending our large tertiary referral lipid clinic in 2008.

Methods: 1283 patients came in 2008, of which 1029 had complete serum lipid and renal profiles. Estimated GFR was calculated for these patients using the 4 variable MDRD formula. The incidence of CKD/proteinuria (albumin creatinine ratio > 2.4 mg/mmol or urinary protein level > 0.06 g/l) was calculated for otherwise healthy hypertensives (n=228), otherwise healthy FH patients (n = 144) and patients with both hypertension and FH (n=51).

Results: The prevalence of CKD/proteinuria in 1029 patients was 11.2% and 19.42% respectively but 4.5% and 12.0% among “background” non hypertensive, non diabetic, non FHs and non familial combined hypercholesterolemic (FCHs) (n = 358). Hypertensives had a significantly raised prevalence of both CKD/proteinuria (17.5% and 21.5). However, FH patients, with significantly elevated total cholesterol levels (mean = 6.04mmol/l, 95% CI: 5.74 - 6.34 mmol/l) relative to background patients (mean = 5.55 mmol/l 95% CI 5.40 - 5.70 mmol/l) did not have an elevated prevalence of CKD/proteinuria.

Conclusions: CKD is common in these patients. Patients with primary dyslipidaemia had a lower prevalence of CKD compared to those with "secondary" dyslipidaemias, or hypertension in addition.

Poster Session

IMPACT OF PLANT STEROLS, FISH OIL OMEGA-3S AND THEIR COMBINATION ON LDL-C AND LDL PARTICLE-SIZE IN INDIAN ADULTS

Shweta Khandelwal[1], I. Demonty[2], P. Jeemon[3], R. Lakshmy[4], R. Mukherjee[1], R. Gupta[4], Y. Singh[4], S.J. Passi[5], D. Prabhakaran[3], S. Reddy[1]

[1]Public Health Foundation of India, New Delhi, India, [2]Unilever Food and Health Research Institute, Unilever R&D Vlaardingen, Vlaardingen, The Netherlands, [3]Centre for Chronic Disease Control, [4]Cardiac Biochemistry, All India Institute of Medical Sciences, [5]Institute of Home Economics, University of Delhi, New Delhi, India

Background and aim: Observational studies suggest greater risk of coronary heart disease in those with higher LDL-C levels and a predominance of small, dense particles. Independent and combined effects of a yoghurt drink (providing 2 g/d PS) and fish-oil capsules (2 g/d w-3s) on LDL-C and LDL particle-size were compared in Indian industrial workers.

Methods: Following a 2-week run-in period, 200 mildly hypercholesterolemic adults aged 35-55 years, were randomized into one of four groups of a 2 x 2 factorial, double-blind, placebo-controlled trial. Subjects (n=178) provided blood samples before and after the 4-week intervention. LDL-C levels were analyzed (quasi Intention-to-treat) using ANCOVA models with relevant baseline measurements as covariates; adjusted means were compared across groups. LDL particle-size was determined by gel electrophoresis of plasma samples. Rank ANCOVA using Mantel-Haenszel zero-correlation test statistic was used to compare the mean value of residuals in the four groups.

Results: Two g/d PS resulted in an overall 4.5% reduction in LDL-C. There was no impact of w-3s on LDL-C; however, the combination resulted in a 4.2% decrease. LDL particle-size was similar in all treatment groups {26.96 (26.88-27.09), 27.03 (26.96-27.15), 26.95 (26.94-27.16) and 27.03(26.98-27.18) nm in placebo, PS active, w-3 active and both active groups respectively}. No significant difference (p=0.8829) was noted in the Mantel-Haenszel test statistic.

Conclusions: The significant reduction in plasma LDL-C concentrations by PS was not paralleled by any beneficial changes in LDL particle-size. However, this could be due to the study being under powered for the latter parameter.

Poster Session

ECCENTRIC ENDURANCE EXERCISE SIGNIFICANTLY LOWERS CRP AND POSTPRANDIAL TRIGLYCERIDES

Philipp Rein[1,2,3], A. Vonbank[1,2,3], C.H. Saely[1,2,3], S. Aczel[1,2], S. Beer[1,2,3], V. Kiene[1], T. Bochdansky[2,4], H. Drexel[1,2,3,5]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Rankweil State Hospital, Departement for Physical Medicine, Rankweil, Austria, [5]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Eccentric exercise (i.e. active resistance to muscle stretching, e.g. with hiking downwards) is less strenuous than concentric exercise; its metabolic effects are largely unknown. We aimed at investigating whether eccentric endurance exercise has anti-inflammatory effects and lowers postprandial triglycerides in healthy subjects without diabetes.

Methods: We conducted a controlled eccentric exercise intervention over a training period of 8 weeks. A total of 93 non-diabetic sedentary subjects were allocated to the intervention group, performing 3 to 5 times per week eccentric endurance exercise by hiking downhill a path in the Austrian alps covering a difference in altitude of 540 meters; for the upward way a cable car was used, where compliance was recorded electronically. The control group included 25 subjects who remained sedentary. Fasting and postprandial metabolic profiles were obtained at baseline and after the two months period.

Results: Compared with baseline, eccentric exercise lowered body mass index (27.7±4.4 vs. 27.4±4.3 kg/m^2; p=0.003) and C-reactive protein (0.27±0.42 vs. 0.23±0.25 mg/dl; p=0.031), whereas both were unchanged in the control group (p=0.053 and p=0.864, respectively). Furthermore, eccentric exercise significantly improved triglyceride tolerance (1959±1331 vs. 1670±1085 $mg*dl^{1}$ h^{1}; p=0.003), whereas triglyceride tolerance was unchanged in the control group (p=0.819).

Conclusion: Eccentric exercise is a promising new exercise modality with favourable metabolic and anti-inflammatory effects.

Poster Session

COMMON CAROTID INTIMA-MEDIA THICKNESS IN HIGH-RISK PATIENTS IN RUSSIA AND EUROPEAN POPULATIONS

I. Sobenin[1,2], V. Myasoedova[1,2], T. Kirichenko[1,2], E. Anisimova[2], S. Castelnuovo[3], M. Amato[3], B. Frigerio[3], A. Ravani[3], **Alexander Orekhov**[1,2], E. Tremoli[3], D. Baldassarre[3], on behalf of the IMPROVE Study Group

[1]Institute of General Pathology and Pathophysiology, [2]Institute for Atherosclerosis Research, Moscow, Russia, [3]Monzino Cardiology Center, Milan, Italy

Background: Russia is characterized by enormously high levels of CVD mortality and morbidity as compared to European populations. A recent European study indicates the existence of a geographical gradient in subclinical atherosclerosis which parallels well the North to South geographical gradient in cardiovascular mortality described in the WHO Europe database.

Aim: To compare the cIMT distribution of a selected high risk populations recruited in Moscow (n=472) with that of the European samples described in the IMPROVE study.

Patients and methods: Asymptomatic men and women (55-79 yrs) with at least three VRFs were eligible for the Study. Different from the IMPROVE Study, ultrasonic scans were focused on the distal centimeter of common carotid arteries only. IMT measurements were performed in Monzino Cardiology Center by a qualified reader using Mat'h software.

Results: The analysis carried out adding the group from Moscow to the IMPROVE study groups revealed that the correlation between the standardized mortality ratio for ischemic heart disease obtained from the WHO Europe database and cIMT is not linear (R-square 0.86, $p < 0.001$ for logarithmic correlation). The mean (95% CI) cIMT value was 0.82 (0.81-0.85) mm in Moscow and 0.73 (0.72-0.74) mm in Milan ($p < 0.001$).

Conclusion: Cardiovascular mortality in Russia is only partially explained by a higher atherosclerosis risk profile; cIMT plays a role as the risk factor for atherosclerotic disease, as well as for the striking difference in cardiovascular mortality between EU and Russia.

Poster Session

ASSOCIATION BETWEEN ATHEROSCLEROSIS AND LUNG FUNCTION - RESULTS FROM INTERNATIONAL TWIN STUDY 2009

David Laszlo Tarnoki[1], A.D. Tarnoki[1], A. Stazi[2], E. Medda[2], R. Cotichini[2], C. Baracchini[3], G. Meneghetti[3], F. Fanelli[4], L. Littvay[5], J. Metneki[6], A.A. Molnar[7], Z. Garami[8], V. Berczi[1], Z. Lazar[9], I. Horvath[9]

[1]Department of Radiology and Oncotherapy, Semmelweis University, Budapest, Hungary, [2]Genetic Epidemiology Unit, National Centre of Epidemiology, Istituto Superiore di Sanità, Roma, [3]Department of Neurosciences, School of Medicine, University of Padua, Padova, [4]Vascular and Interventional Radiology Unit, Department of Radiological Sciences, La Sapienza University of Rome, Roma, Italy, [5]Central European University, [6]Genetic Epidemiology Unit, National Centre of Epidemiology, [7]Research Group for Inflammation Biology and Immunogenomics of Hungarian Academy of Sciences and Semmelweis University, Semmelweis University, Budapest, Hungary, [8]The Methodist Hospital DeBakey Heart and Vascular Center, Houston, TX, USA, [9]Department of Pulmonology, Semmelweis University, Budapest, Hungary

Background: An association between reduced pulmonary function and an increased risk of atherosclerotic vascular events has been reported but the underlying mechanisms are still unknown. So far no twin studies have examined the cross-sectional association between subclinical atherosclerosis and pulmonary function.

Objective: To estimate association of arterial stiffness and lung function and to assess heritability and environmental effects on these parameters.

Subjects and methods:128 Italian (70 monozygotic /MZ/ and 58 dizygotic /DZ/), 50 American (47 MZ, 3 DZ) and 86 Hungarian (62 MZ, 24 DZ) twin pairs were included in this classical twin study as part of International Twin Study 2009. TensioMed Arteriograph and MIR Minispir spirometer were used for measuring arterial stiffness and lung function, respectively.

Results: Based on all 264 sample pairs (age ys 50.5±15.4; mean±SD), age and gender-corrected heritability of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were 0.74 and 0.75 ($p< 0.01$). Shared and unshared environmental effects were found to be 0, 0.26 ($p< 0.01$) and 0, 0.25 ($p< 0.01$) for FVC and FEV_1. Pearson correlation coefficient (PCC) between FVC and Augmentation index on brachial artery (Aixbra) and Pulse Wave Velocity on aorta (PWVao) were -0.42 ($p< 0.001$) and -0.29 ($p< 0.001$). PCCs between FEV_1 and Aixbra and PWVao were found to be -0.47 ($p< 0.001$) and -0.38 ($p< 0.001$), respectively.

Conclusions: The lung function parameters of FVC and FEV_1 are strongly heritable and are associated with arterial stiffness. The observed relationship can aid to understand the background of vascular changes in different airway diseases.

Poster Session

IS HIGH ANKLE-BRACHIAL INDEX PREDICTOR OF INCREASED CARDIOVASCULAR RISK? THE CZECH POST-MONICA STUDY

Peter Wohlfahrt[1], D. Palous[2], M. Ingrischova[3], A. Krajcoviechova[1], J. Seidlerova[4], J. Bruthans[1], M. Galovcova[1], V. Adamkova[1], J. Filipovsky[4], R. Cifkova[1], The Czech post-MONICA Study Group

[1]Department of Preventive Cardiology, [2]Department of Radiology, Institute for Clinical and Experimental Medicine, [3]2nd Medical Department-Clinical Department of Cardiology and Angiology, 1st Medical Faculty, Charles University, Prague, [4]Center of Preventive Cardiology, 2nd Department of Internal Medicine, Charles University, Pilsen, Czech Republic

Ankle brachial index (ABI) has increasingly been used in general practice to identify patients with low ABI at high cardiovascular risk. However there is no consensus on the clinical significance of high ABI. The aim of our study was to compare large artery stiffness as a marker of cardiovascular risk in patients with low (< 1.0), normal (1.0-1.4) and high ABI (>1.4).

Methods: We examined 911 patients from the Czech post-MONICA study (a randomly selected 1% representative population sample, mean age 54±13.5 years, 47% of men). ABI was measured using a handheld Doppler and aortic pulse wave velocity (aPWV) using the Sphygmocor device.

Results: Of the 911 patients, 28 (3.1%) had low ABI and 23 (2.5%) high ABI. There was a U-shaped association between aPWV and ABI. aPWV was significantly higher in patients with low and high ABI compared with normal ABI group (11.1±2.8 vs. 8.3±2.3, p< 0.001; 10.8±2.5 vs. 8.3±2.3, p< 0.001) and it did not differ between patients with high and low ABI (11.1±2.8 vs. 10.8±2.5, p=0.86). In the logistic regression analysis aPWV together with glucose level, male sex and history of deep venous thrombosis were independent predictors of high ABI.

Conclusion: This is the first study showing increased aortic PWV in patients with high ABI pointing to increased cardiovascular risk in this group.

Poster Session

PREMATURE TREATMENT EFECTS OF METABOLIC SYNDROME AS THE PREDICTOR OF DIABETES MELLITUS TYPE 2

Tea Gamezardashvili, N. Kipshidze

Department of Heart Ischemic Disease, National Center of Therapy, Tbilisi, Georgia

Insulin resistance and hyperglycemia are very frequent disorders. They are describing as the dominant risk factors for Diabetes Mellitus type 2 and cardiovascular diseases.

We investigated 188 individuals with 3 or more risk factors of Metabolic Syndrome (MS). MS was defined according to the IDF criteria. They consider themselves practically healthy before the investigation. After having performed OGTT, impaired glucose regulation (IGR) was observed in 38.3% and already existing asymptomatic type 2 diabetes was observed in 14.9%. 72 patients were randomly assigned and divided into two groups.

The first group patients were given recommendations about life style modification. The second group patients were administered the following medicines: ACE inhibitor, statin (Atorvastatin 20mg, Simvastatin 20mg or Rosuvastatin 10mg) and Metformine (1000 mg/day); also were given recommendations about life style modification. OGTT was once more performed in both groups after six months.

In the first group in 8 patients (22.2%) glucose metabolism regulated, in 6 patients (16.7%) developed type 2 diabetes and the condition of 22 patients (61.1%) didn't change.

In the second group In 17 patients (47.2%) glucose metabolism regulated, only in 3 patients (8.3%) developed type 2 diabetes and the condition of 16 patients (44.5%) didn't change.

Conclusions: Metabolic Syndrome as the cluster of Cardio Vascular Diseases risk factors is the predictor of type 2 diabetes; early therapy with ACE inhibitor, statin and Metformine contributes the discontinuation of the above mentioned pathologic processes and prevents the development of type 2 diabetes.

Poster Session

PROGNOSTIC VALUE OF ENDOTHELIUM-DEPENDENT VASODILATATION AND PULSE WAVE VELOCITY IN YOUNG HYPERTENSIVE PATIENTS

Tatyana Pronko

Grodno State Medical University, Grodno, Belarus

Endothelial dysfunction and increased arterial stiffness are two indicators of subclinical atherosclerotic disease. The aim of the study was to observe if there is a relationship between endothelial dysfunction and arterial stiffness in young hypertensive patients.

Methods: The study population included 52 patients with arterial hypertension (28 men and 24 women), average age 36.5±2.1 years and 47 healthy volunteers (33 men 14 women), average age 36.0±1.43 years. Endothelial function was measured by strain-gauge plethysmography. The forearm blood flow (FBF) was measured during reactive hyperemia to test endothelium-dependent vasodilatation, and after sublingual nitroglycerin administration - to test endothelium-independent vasodilatation. The criterion for endothelial dysfunction was less than 10% increase of the forearm blood flow after reactive hyperemia.

Carotid brachial pulse wave velocity (PWV) was measured noninvasively.

Results: FBF during reactive hyperemia in patients with hypertension (15.03±2.1%) was significantly less than that in control subjects (22.30±1.5%, $p< 0.05$). Endothelial dysfunction was found in 32.6% of the patients. The increases in FBF after nitroglycerin were similar between the hypertensive and normotensive subjects.

The mean carotid brachial PWV in hypertensive patients was 8.81±0.92 m/s, what is significantly higher compared with healthy persons 5.94±0.42 m/s ($p< 0.01$). FBF during reactive hyperemia and carotid brachial PWV were negatively correlated in the both examined groups ($r=-0.39$; $p< 0.05$).

Conclusion: In young hypertensive patients, FBF and PWV measurements are important tools for evaluation of arterial wall remodeling and can be used as valid early markers of atherosclerosis in cardiovascular risk prediction.

Poster Session

SERUM URIC ACID, BUT NOT RS7442295 POLYMORPHISM OF SCL2A9 GENE, PREDICTS TOTAL AND CARDIOVASCULAR MORTALITY IN SEVERE CORONARY ARTERY DISEASE

Nicola Martinelli[1], L. Annarumma[1], D. Girelli[1], G. Malerba[2], F. Pizzolo[1], S. Friso[1], L. Consoli[1], T. Illig[3], P.F. Pignatti[2], R. Corrocher[1], O. Olivieri[1]

[1]Department of Clinical and Experimental Medicine, [2]Department of Mother and Child and Biology-Genetics, Section of Biology and Genetics, University of Verona, Verona, Italy, [3]Institute of Epidemiology, GSF-National Research Center for Environment, Neuherberg, Germany

Background: High levels of serum uric acid (SUA) have been associated with cardiovascular events in several studies, but the role of hyperuricemia as independent prognostic predictor of cardiovascular mortality is still matter of debate. The aim of the current study were: 1) to examine the predictive value of SUA for mortality in the setting of secondary prevention of coronary artery disease (CAD); 2) to evaluate possible associations between mortality and rs7442295 polymorphism of SCL2A9 gene, that has been related to SUA in recent genome-wide association studies.

Methods: A cohort of 703 patients with angiographically proven CAD was prospectively followed for a median period of 57 months. The large majority of them (92.5%) underwent coronary revascularization.

Results: During the follow-up, 116 (16.5%) out of 703 patients died, with 83 events (11.8%) attributed to cardiovascular causes. After adjustment for all the other predictors of mortality at univariate analysis (i.e. age, myocardial infarction history, ejection fraction, diabetes, hs-CRP and creatinine, statin, beta-blockers, and allopurinol therapy), elevated SUA levels (≥0.41 mmol/l - the 75° percentile) significantly predicted both total and cardiovascular mortality (HR for total mortality 1.87 with 95%CI 1.05-3.34; HR for cardiovascular mortality 2.09 with 95%CI 1.03-4.25). Although rs7442295 was an independent predictor of SUA (standardized beta-coefficient for the G allele -0.100, *P*=0.008 by adjusted linear regression), it was not associated with total or cardiovascular mortality.

Conclusions: Basal concentrations of SUA levels ≥0.41 mmol/l in CAD patients independently predicted total and cardiovascular mortality, whereas no association was found between rs7442295 polymorphism and mortality.

Poster Session

SMOKING BEHAVIORS AFTER ACUTE CORONARY SYNDROMES (ACS) IN ADULT MALE SMOKERS

Ioannis Tzanogiorgis, E. Nikolaou, I. Kogias

Department of Cardiology, General Hospital of Karditsa, Karditsa, Greece

Objective: To examine changes in smoking behaviors after experiencing an ACS as well as the factors influencing the smoking status.

Methods: Male survivors of ACS, all smokers until the day of the event, who attended the outpatients' cardiological department in a period of three months, completed a questionnaire including demographic, medical and smoking status data. The statistical analysis was performed by the program SPSS v13.0.

Results: We examined 51 post-ACS patients. The mean age at the event was 51.3 (±9.7) years and the median pack-years were 52 (8-172). 84% of the patients had experienced AMI, while the rest had unstable angina with invasive treatment. In total, 13.7 % had conservative treatment, 55% had undergone PTCA and the rest CABG. 59% of the patients quitted smoking while 23.6% stopped smoking for a median period of 4 (1-42) months.

There wasn't found any statistically important correlation between smoking cessation and level of education ($p=0.62$). There wasn't found any statistically important correlation between smoking cessation and type of event ($p=0.82$), kind of treatment ($p=0.76$) or patient's age at the time of the event ($p=0.76$).

Conclusions: A hospital admission for a serious cardiac event should offer a unique opportunity for smoking cessation. Nevertheless, stressful events like AMI and the invasive treatments that follow seem not to motivate patients to quit smoking. Doctors should promote smoking cessation more vigorously in these patients.

Poster Session

"PARAOXONASE (PON1) GLN, ARG 192 POLYMORPHISM" AN INDEPENDENT RISK FACTOR FOR CORONARY ARTERY DISEASE IN NORTH-WEST INDIAN POPULATION

Nidhi Gupta[1], S. Singh[2], K.D. Gill[1]

[1]Biochemistry, [2]Internal Medicine, Post Graduate Institute of Medical Education & Research, Chandigarh, India

Aim: PON1 is HDL-C associated enzyme which protects LDL-C from peroxidation. Two common polymorphisms of PON1 gene, Q192R and L55M, determine inter-individual variation in PON1 activity. As North West Indian punjabi's have high incidence of CAD and no information is available regarding these, we investigated the role of these polymorphisms in this population.

Methods: We looked at the genotype distributions of these two polymorphisms in 350 angiographically proven CAD patients and compared it with 300 healthy controls. The correlation of these polymorphisms with PON1/ARE activity and with lipid profile was also determined.

Results: The PON1 192RR genotype and the frequency of the R allele were significantly higher (P< 0.0001) in CAD patients as compared to controls. However, no significant difference was observed for L55M polymorphism. On multiple regression analysis involving all factors involved in CAD, the 192RR genotype remained independently associated with a more than 3-fold increase in the risk of it (OR: 3.52, 95%CI: 0.97-6.93). The PON1 activity was significantly higher (P< 0.0001) in RR genotype followed by QR and QQ genotype in both populations. However L55M polymorphisms had no correlation with PON1 activity in any group. The ARE activity was not affected by Q192R and L55M polymorphisms. The PON1 192 and 55 polymorphisms did not consistently influence the serum lipid profile in either population.

Conclusion: The PON1 activity in our study population was affected by Q192R polymorphism and 192RR genotype was independently associated with an increased risk of CAD. ARE activity was not affected by PON1 polymorphisms.

Poster Session

GAMMA-GLUTAMYL-TRANSFERASE, GLUTAMATE-PYRUVATE-TRANSAMINASE, AND THE GLUTAMATE-PYRUVATE-TRANSAMINASE / GLUTAMATE-OXALACETATE TRANSAMINASE RATIO ARE SIGNIFICANTLY REDUCED BY EIGHT WEEKS OF ECCENTRIC ENDURANCE EXERCISE

Christoph H. Saely[1,2,3], P. Rein[1,2,3], A. Vonbank[1,2,3], S. Beer[1,2,3], V. Kiene[1], S. Aczel[1,2], T. Bochdansky[2,4], H. Drexel[1,2,3,5]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Rankweil State Hospital, Departement for Physical Medicine, Rankweil, Austria, [5]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Elevated serum gamma-glutamyl-transferase (GGT) and glutamate-pyruvate-transaminase (GPT) as well as an elevated glutamate-pyruvate-transaminase / glutamate-oxalacetate transaminase (GPT/GOT) are associated with the metabolic syndrome and, in some studies, with an increased cardiovascular risk. We hypothesised that eccentric endurance exercise lowers these parameters of liver function.

Material and methods: Over a training period of 8 weeks, 51 healthy non-diabetic subjects (16 men and 35 women, mean age 50.3 years) 3 to 5 times per week performed eccentric endurance exercise by hiking downhill a path in the Austrian alps covering a difference in altitude of 540 meters; for the upward way a cable car was used, where compliance was recorded electronically.

Results: GGT (from 51 ± 90 at baseline to 41 ± 60 mg/dl after 8 weeks of eccentric endurance exercise; p= 0.001), GPT (from 35 ± 30 to 30 ± 17 mg/dl; p = 0.006) and, most strongly, the GPT/GOT ratio (from 1.18 ± 0.39 to 1.00 ± 0.32; p < 0.001) were significantly decreased with 8 weeks of eccentric endurance exercise.

Conclusion: GGT, GPT, and the GPT/GOT ratio even in apparently healthy individuals are significantly reduced by 8 weeks of eccentric endurance exercise. This modestly strenuous exercise modality therefore may me represent a promising treatment option for metabolic syndrome related non-alcoholic fatty liver disease.

Poster Session

ATRIAL FIBRILATION AS CONTRIBUTING FACTOR TO STROKE EPIDIOMIOLIC STUDY IN THE GREEK POPULATION

Ioannis Kanonidis[1], K. Martiadou[1], G. Rompolas[1], N. Sailer[2], S. Tzotzis[2], C. Kagiadaki[1], D. Eclisiarhos[1], G. Sakantamis[1]

[1]2nd Department of Cardiology, Aristotle University of Thessaloniki, [2]1st Department of Internal Medicine, Hippokration Hospital, Thessaloníki, Greece

Introduction: The study aims to evaluate the incidence of Atrial Fibrillation (AF) and its possible pathophysiologic implications in patients with stroke.

Methods: The study included all patients hospitalized for stroke during a period of 2 years in our department. All patients had a thorough investigation on admission including CT scan, ECG, Echo (transthoracic and transesophageal), e.tc.

Results: 539 patients, 306 men and 233 women of mean age 74.9 years were hospitalized for stroke. Of those 501 had a thromboembolic episode and 38 a hemorrhagic one. AF was noticed on admission in 138(25.6%) patients, 66 men (21.6%) and 72 women (30.8%). Only 2 patients with AF had a hemorrhagic stroke, both under optimal anticoagulation therapy. Of the 136 AF patients with thromboembolic stroke 84 (62%) presented with TIA and 52 (38%) with permanent stroke. Of those 22 had a new diagnosis of AF, 32 paroxysmal AF of whom only 12 were on anticoagulants. Of the remaining 82 patients with permanent AF, 9 had no anticoagulation in 32 anticoagulation was suboptimal and only in 41 was found to be optimal. Intracardiac thrombi were detected in 4 patients while indirect signs were noticed in another 16 patients with Esophageal Echo.

Conclusion: AF is a contributing factor in almost one forth of stroke cases. It is almost exclusively related to thromboembolic events.

It is more frequent in women than in men. Its probable involvement in the pathogenesis of stroke, it is invoked inabout half of the cases and is associated with suboptimal anticoagulation.

Poster Session

SUBCLINICAL ATHEROSCLEROSIS DETERMINED BY PULSE WAVE VELOCITY IN OVERWEIGHT AND OBESE RURAL FILIPINO SUBJECTS

Helen Marcoyannopoulou Fojas[1], E.B. Barrios[2]

[1]Philippine Department of Science and Technology, Taguig City, [2]School of Statistics, University of the Philippines, Quezon City, Philippines

Purpose: To show by Pulse Wave Velocity (PWV) that pathological arterial wall changes occur both in obese and overweight individuals.

Method: PWV determined with the BPULS apparatus using left external carotid and left dorsalis pedis arteries as “central” and “peripheral” points respectively. Arterial pulses picked up by sensors and recorded on computer simultaneously with single lead ECG. Time delay between two pulses measured. Shorter time indicates decreased arterial elasticity.

Waist circumference (WC) measured. Weight and height for BMI.

Materials: Total - 950 asymptomatic Filipinos in rural areas studied. Females - 553; Males - 397. Age range - 17 - 84 years

Females : WC< 80 cm. - 145, WC> 80 cm. -408; Males: WC < 90 cm. - 226, WC> 90 cm. - 171,

By BMI: Underweight -102; Normal- 481; Overweight- 283; Obese -84.

Results: PWV Time (sec.) - Females with WC < 80 cm. - 0.1456, >80 cm.- 0.1290; Males with WC < 90 cm. - 0.1506, >90 cm. - 0.1331. $p < 0.03$

PWV Time (sec,) - Underweight - 0.1544; Normal - 0.1404; Overweight - 0.1289; Obese - 0.1257. $p < 0.03$

Discussion: Decreased arterial wall elasticity is associated with endothelial dysfunction. We showed decreased arterial elasticity in subjects having increased WC and who are obese and even just overweight, These subjects have high risk to develop CVD. Overweight individuals should reduce as it is easier before they become obese.

Conclusion: Subjects with increased WC and high BMI should reduce to avoid CVD.

Poster Session

THE ASSOCIATION BETWEEN VARIATION IN *NEGR1* GENE AND BODY-WEIGHT IS MODULATED BY PHYSICAL ACTIVITY IN A HIGH-RISK MEDITERRANEAN POPULATION

Mercedes Sotos-Prieto[1,2], C. Ortega-Azorín[1,2], P. Carrasco[1,2], J.V. Sorli[1,2], P. Guillem-Saiz[1,2], J.I. Gonzalez[1,2], D. Corella[1,2]

[1]Genetic and Molecular Epidemiology Unit, University of Valencia, Valencia, [2]CIBER Fisiopatología de la Obesidad y Nutrición, Santiago de Compostela, Spain

Introduction: The neuronal growth regulator 1 (*NEGR1*) gene (1p31), is a newly identified gene associated with obesity phenotypes. It is involved in neural development. Physical activity has also been reported to interact with other novel obesity genes in modulating the associations.

Objective: To study the association between the rs2568958 in *NEGR1* gene with body-weight and other related variables in a high-risk Mediterranean population as well as its modulation by physical activity.

Material and methods: We studied 1050 subjects with high cardiovascular risk (aged: 67±6years) participating in the PREDIMED-Valencia (PREvención con Dieta MEDiterránea). Anthropometric, clinical, genetic and life-style variables were obtained. The rs2568958 polymorphism was determined and physical activity was assessed by questionnaire.

Results: Participants had a high body-mass index (mean 31Kg/m^2) as well as a high prevalence of diabetes (45%) and hypertension. Prevalence of rs2568958 was AA:41.2%; AG:40.6%; GG:10.5%. Homozygous AA subjects had higher body-weight (p=0.037). Waist circumference and BMI were also higher in AA subjects, without reaching statistical significance (p=0.074 and p=0.176 respectively). Physical inactivity increased the effects of the polymorphism. Sedentary subjects with the obesity-risk genotyped had significantly higher body-weight (AA 85.5 ± 10.9 *vs* AG+GG: 74.3±11.1 Kg; p=0.015), whereas in active subjects these differences were not found (AA: 77.0±12.2 *vs* AG+GG: 76.3±11.7 Kg; p= 0.613). Furthermore, the NEGR1 AA was associated with higher hypertension, which remained significant even after adjustment for BMI (adjusted OR 2.03 [95% CI: 1.4-2.9]).

Conclusion: The effect of the NEGR1 AA genotype on body-weight is modulated by physical activity in this Mediterranean population

Poster Session

DAILY INTAKE OF A DAIRY DRINK ENRICHED WITH OMEGA-3 (EPA+DHA) AND OLEIC ACID IMPROVES CARDIOVASCULAR MARKERS IN HEALTHY POSTMENOPAUSAL WOMEN

Juristo Fonolla[1], M. Diaz-Ropero[1], A. Geerlings[1], J. Marti[2], E. Lopez-Huertas[3]

[1]Human Nutrition, Puleva Biotech, [2]Puleva Food SA, [3]Estación Experimental Zaidín, Consejo Superior Investigaciones Científicas (CSIC), Granada, Spain

Aims: To investigate the effects produced by a 1-year consumption of an EPA+DHA and oleic acid enriched dairy drink in cardiovascular risk markers of postmenopausal women.

Methods: A randomised, controlled, double-blind design was used in the intervention. 117 postmenopausal women (59,7±5,8 years) with low-moderate cardiovascular risk were included in the study. They were randomly distributed into two study groups: the Control group (CG, n=54, 61,7±5,7 years) that consumed 500 mL/day of standard semi-skimmed milk and the supplemented group (SG, n=63, 57,9±5,2 years) which consumed 500 mL/day of a low-lactose skimmed milk enriched with 40 mg/100 mL of EPA +DHA, 0.54 g/100 mL oleic acid, 0.5 g/100 mL of soluble fiber, minerals and vitamins A, B6, C, D, E and folic acid (Puleva Digestiva®).

Results: 12-month consumption of the enriched dairy drink (SG) produced reductions in total cholesterol (-6%), LDL-cholesterol (-10%), the total cholesterol/HDL-cholesterol ratio (-6%), glucose (-5%) and C-reactive protein (-28%). Significant increases in serum folate (75%), erythrocyte folate (53%), vitamin B6 (58%), and a decrease in the ratio arachidonic acid/EPA (-41%) were also observed. Plasma triglycerides showed a non-significant reduction (-6%) and homocysteine remained unchanged at the end of the study. Significant reductions in HDL-cholesterol (-5%) and serum folate (-12%) were the only changes observed in the control group.

Conclusions: The dietary supplementation with a low-lactose dairy drink enriched with omega-3 fatty acids (EPA+DHA), oleic acid, minerals and vitamins improved the cardiovascular risk profile, and its regular consumption may be useful as nutritional support in postmenopausal healthy women.

Poster Session

EVEN SMALL DECREASE IN WEIGHT TROUGH LIFE STYLE MODIFICATIONS CAN IMPROVE RISK FACTORS ASSOCIATED TO ATHEROSCLEROSIS

Rosa Santos[1,2], J. Freitas[3], M. Macedo[4], I. Rebelo[2]

[1]Internal Medicine, Hospital S. Joao, [2]Biochemistry, University of Pharmacy, [3]Cardiology, Hospital S. Joao, [4]School of Medicine, Porto University, Porto, Portugal

Introduction: In obesity population studies show that a reduction as little as 10% of baseline weight improved the control of some biomarkers linked with atherosclerosis.

Objectives: Evaluation the effect of weight reduction on arterial blood pressure, metabolic and inflammatory biomarkers.

Methods: Seventy-four patients with essential hypertension and obesity (BMI >30kg/m^2) were observed in our hypertension clinic at an university hospital (14 men mean age 48.6±6.4 and 60 women mean age 44.3±10.2). The first clinical evaluation included blood pressure anthropometric evaluation and fast biochemical measurement. Patients were then prescribed with hypocaloric diet adjusted to age, gender, physical activity, social and/or professional lifestyle. The patients were distributed in accordance with their risk factors associated with obesity and metabolic indexes. Patients were under close follow up during 6 years in order to assess anthropometric parameters, blood pressure and biochemical analysis, to adjust calories in their diet. All patients were under antihypertensive drug that did not changed during follow-up.

Results: First evaluation versus Last evaluation: Weight(Kg) 88.2±11.5vs79.3±11.6 p< 0.05;BMI(Kg/m^2) 32.3±4.8vs29.1±4.9 p< 0.04;Weight Fat 35.9±13.7vs30.6±10.7 p< 0.05;Conicity Index 0.652±0.151vs0.544±0.132 p< 0.03;Waist Circumference(cm) 99.4±13.0vs89.1±11.4 p< 0.03;HR (bpm) 80.3±9.6vs69.4±10.7 p< 0.01;SBP (mmHg) 145.4±15.2vs119.5±18.6 p< 0.01;DBP 92.1±11.5vs75.3±13.6 p< 0.01;Glucose(mg/dL) 119.0±15.0vs98.5±16.3 p:ns;Insulin(µmol/L) 18.4±6.4vs10.5±4.2 p< 0.01;Total Cholesterol(mg/dL) 236.8±30.0vs195.4±28.3 p:ns;Triglycerides(mg/dL)180.2±78.5vs 112.4±20.2 p< 0.01;LDL-C (mg/dL) 128.3±16.3vs111.7±15.9 p< 0.04;Homocysteine(µmol/L) 10.7±1.8vs7.2±1.6 p< 0.05;PCR(mg/L) 0.285±0.158vs0.112±0.100 p:ns;IL-6(ng/ml) 18.8±10.1vs16.9±10.8 p:ns;Myeloperoxidase(ng/ml) 77.3±21.3vs59.37±20.4 p:ns.

Conclusions: This study shows that even a small weight reduction in obesity populations improved the risk factor profile to cardiovascular diseases such as blood pressure homocysteine, insulin, glucose, lipids and some inflammatory biomarkers.

Poster Session

MODERATE PHYSICAL ACTIVITY IS ASSOCIATED WITH LOWER APOB/APOA-I RATIOS INDEPENDENTLY OF OTHER RISK FACTORS IN HEALTHY MIDDLE-AGED MEN

Caroline Schmidt, C.J. Behre, G. Bergström

Department of Molecular and Clinical Medicine, Wallenberg Laboratory for Cardiovascular Research, Institution of Medicine, Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden

Introduction: LDL has for long been recognized as the primary risk factor for CVD. However, new data favour apoB and apoA-I as risk factors with a higher predictive value than conventional lipids. Physical activity has limited impact on LDL levels, but recent studies indicate that physical activity lowers apoB and/or increases apoA-I.

Objective: The aim of the present paper was to investigate how leisure time physical activity relates to the serum apoB/apoA-I ratio in a group of middle-aged men.

Material and methods: We recruited 391 clinically healthy 58-year-old men from the general population of Göteborg, Sweden. The level of leisure time physical activity was assessed by a self-administered, three-scale questionnaire, apoB, apoA-I and conventional lipids were measured.

Results: Compared to a sedentary lifestyle, moderate physical activity was associated with a decreased apoB/apoA-I ratio (0.87±0.24 vs.1.01±0.28, $p< 0.05$) and increased apoA-I levels (1.43±0.22 g/L vs.1.30±0.20 g/L, $p< 0.05$). A co-variate analysis with smoking, systolic blood pressure, waist circumference, alcohol intake and psychological well-being, showed that physical activity was associated with apoB/apoA-I ratio ($p< 0.05$) also after adjustment. No association between physical activity and LDL-cholesterol was observed ($p=0.30$)

Conclusion: Physical activity, showed favourable associations with the apoB/apoA-I ratio and apoA-I levels in this cohort of healthy middle-aged men.

Further, physical activity was associated with lower levels of apoB/apoA-I ratio independently of other risk factors. Importantly, these associations were seen already at moderate levels of physical activity supporting the notion that some activity is better than none in terms of health benefits.

Poster Session

PERSISTENT LIPID ABNORMALITIES IN STATIN TREATED PATIENTS IN SPAIN. THE DYSLIPIDEMIA INTERNATIONAL SURVEY STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], C. Guijarro[3], E. Alegria[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hospital Clinico Univesitario de Santiago de Compostela, Santiago de Compostela (La Coruña), [3]Hospital de Alcorcon, Alcorcon (Madrid), [4]Clinica Universitaria de Pamplona, Pamplona (Navarra), [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Intoduction: Despite of statin therapy,the incidence of cardiovascular (CV) events is high.LDL-C goal is sometimes not reached and other lipid abnormalities can also contribute to CV risk (CVR).The objective of DYSIS was to assess the prevalence of lipid abnormalities (LDL-C, HDL-C and triglycerides) in patients treated with statins.

Methods: Analysis of 3710 Spanish patients included in DYSIS, (22063 patients in Europe and Canada)≥45 year-old treated with statins.We used the ATP-III recommendations to define patient´s risk,LDL-C goal and the normality or not of the HDL-C and triglycerides (TG).

Results: In 3710 patients (18.6% current smokers,35.1% obese,69.0% with hypertension,39.0% with diabetes mellitus,35.7% with established CV disease,71.2% high-risk patients)LDL-C was not at goal in 63.5% of patients,more frequent in high CVR patients(58.8% [goal< 100 mg/dl],89.8% considering< 70 mg/dl) and moderate-risk (48.9% [goal< 120 mg/dl]) vs. low risk patients(21.3% [goal < 160 mg/dl])(p< 0.001).Low HDL-C was present in 29.8% of the patients and in 34.8%,37.1% and 2.1% of those at high,moderate or low CV risk,respectively.TG≥150 mg/dl in 37.6% and in 40.7%, 42.0% and 20.8%(high, moderate or low CV risk, respectively).The 36.8% had two or more abnormalities(6.4% abnormal LDL-C+low HDL-C, 13.1% abnormal LDL-C+TG, and 9.7% had abnormal values of the three parameters).Only 28.4% of patients had the three lipids within the recommended/normal range.

Conclusions: Despite statin therapy,a high percentage of patients show lipid abnormalities,not only abnormal LDL-C but also low HDL-C and/or abnormal TG,especially those at higher CVR.An integrated approach to the treatment of dyslipidaemia may be of interest to reduce the CVR complications even in patients treated with statins.

Poster Session

RELATIONSHIP BETWEEN ALCOHOL INTAKE, HDL-CHOLESTEROL AND CARDIOVASCULAR RISK-FACTORS IN THE URBAN PARIS-ILE-DE-FRANCE COHORT: IS THE CARDIOPROTECTIVE ACTION OF ALCOHOL A MYTH?

Boris Hansel[1], F. Thomas-Jean[2], B. Panier[2], K. Bean[2], A. Kontush[1], M.J. Chapman[1], L. Guize[2], E. Bruckert[3]

[1]INSERM Unit 939, [2]IPC, [3]APHP, Paris, France

Objective: Observational studies document inverse relationship between cardiovascular disease (CVD) and moderate alcohol intake. Causal role for alcohol in cardioprotection remains however uncertain as such protection may partly arise from confounders and misclassification. The aim of our study was to evaluate potential confounders which may contribute to the putative cardioprotection by alcohol.

Methods: We evaluated clinical and biological characteristics, including CV risk factors and health status, of 149,773 subjects undergoing examination in our Center for CVD Prevention (The Urban Paris-Ile-de-France Cohort). Subjects were divided into four groups according to alcohol consumption: never, low (≤10g/day), moderate (10 to 30 g/day) and high (>30g/day); former drinkers were analysed as a separate group.

Results: After adjustment for age, moderate male drinkers were more likely to display clinical and biological characteristics associated with lower CV risk, including low BMI, heart rate, pulse pressure, fasting triglycerides, fasting glucose, stress and depression scores together with superior subjective health status, respiratory function, social status and physical activity. Moderate female drinkers equally displayed low waist circumference, blood pressure and fasting triglycerides and LDL-cholesterol. Alcohol intake was strongly associated with plasma HDL-cholesterol in both sexes. Multivariate analysis confirmed that moderate and low drinkers displayed better health status than never drinkers. Importantly, few factors were causally related to alcohol intake.

Conclusion: Moderate alcohol drinkers display a more favourable clinical and biological profile, consistent with lower CV risk as compared to non-drinkers and heavy drinkers. Moderate alcohol consumption may therefore represent a marker of higher social level, superior health status and lower CV risk.

Poster Session

ADHERENCE TO THE MEDITERRANEAN DIETARY PATTERN IS INVERSELY ASSOCIATED WITH SMALL DENSE LOW-DENSITY LIPOPROTEIN (SDLDL) PHENOTYPE B: THE ATTICA STUDY

Christina-Maria Kastorini[1], D.B. Panagiotakos[1], C. Chrysohoou[2], C. Pitsavos[2], J. Skoumas[2], S. Vellas[2], M. Kambaxis[2], C. Stefanadis[2]

[1]Department of Nutrition Science - Dietetics, Harokopio University, [2]First Cardiology Clinic, School of Medicine, University of Athens, Athens, Greece

Objective: Lipoprotein phenotype B is characterized by a predominance of small dense LDL particles and is associated with increased risk for developing coronary heart disease. The aim of the present work is to examine the relationship between adherence to the Mediterranean dietary pattern and the level of small dense LDL-cholesterol.

Methods: The ATTICA study is a population-based cohort that has randomly enrolled 1528 men and 1514 women (aged > 18 years old), stratified by age - gender, from the greater area of Athens, during 2001-2002. Adherence to Mediterranean diet was assessed through the MedDietScore (theoretical range 0-55). The LDL-cholesterol/LDL-apo-B ratio was calculated using the formula (0.94chol-0.94HDL-0.19TG)/(apo-B-0.09chol+0.09HDL-0.08TG). Lower levels of this ratio indicate the presence of small dense LDL.

Results: After controlling for several potential confounding factors, participants in the lowest tertile and in the second tertile of the MedDietScore (i.e., < 27, lower adherence to the Mediterranean diet), had lower LDL-cholesterol/LDL-apo B ratio, compared with those in the highest tertile (B=-0.063, p=0.012 and B=-0.049, p=0.009, respectively). In addition multiple logistic regression analysis, showed that participants following very closely the Mediterranean dietary pattern have 36% (95%CI: 0.42-0.96) lower likelihood of having levels of the LDL-cholesterol/LDL-apo-B ratio below the median, i.e., 1.35, compared with those who follow a more western diet.

Conclusions: Adherence to the Mediterranean dietary pattern has a protective effect regarding the presence of the more atherogenic small dense LDL particles that are considered a major risk factor for cardiovascular disease.

Poster Session

ATHEROSCLEROSIS AND BODY COMPOSITION. FINDINGS OF INTERNATIONAL TWIN STUDY 2009

Adam Tarnoki[1], D.L. Tarnoki[1], A. Stazi[2], E. Medda[2], R. Cotichini[2], C. Fagnani[2], L. Nisticò[2], P. Lucatelli[3], E. Boatta[3], C. Zini[3], G. Meneghetti[4], C. Baracchini[4], J. Osztovits[5], G. Jermendy[5], L. Littvay[6], J. Metneki[7], K. Karlinger[1], A.A. Molnar[8], F. Fanelli[3], Z. Garami[9], V. Berczi[1]

[1]Department of Radiology and Oncotherapy, Semmelweis University, Budapest, Hungary, [2]Genetic Epidemiology Unit, National Centre of Epidemiology, Istituto Superiore di Sanità, Roma, [3]Vascular and Interventional Radiology Unit, Department of Radiological Sciences, La Sapienza University of Rome, Rome, [4]Department of Neurosciences, School of Medicine, University of Padua, Padova, Italy, [5]Bajcsy Zsilinszky Hospital, III. Department of Internal Medicine, Semmelweis University, [6]Central European University, [7]Department of Human Genetics, National Center of Epidemiology, [8]Research Group for Inflammation Biology and Immunogenomics of Hungarian Academy of Sciences and Semmelweis University, Semmelweis University, Budapest, Hungary, [9]Methodist DeBakey Heart and Vascular Center, Houston, TX, USA

Background: A potential application of bioimpendance analysis (BIA) in the clinical prediction and diagnosis of cardiovascular disease has been reported but the underlying mechanisms are still unknown.

Purpose: To estimate influence of body composition on arterial stiffness moreover heritability and environmental effects using a twin sample.

Subjects and methods: 126 Italian, 48 American and 82 Hungarian twin pairs were included in this study as part of International Twin Study 2009. TensioMed Arteriograph and OMRON BF500 (by BIA) were used for measuring arterial stiffness and body composition.

Results: Based on 512 samples (342 monozygotic /MZ/, 170 dizygotic /DZ/; mean age 50.9±15.2 years), mean Augmentation index (Aixbra) and Pulse Wave Velocity on aorta (PWVao) indicated -16.4±32.3% and 9.008±2.4 m/s. Age-adjusted intraclass correlation of Aixbra and PWVao were 0.65 (95% CI, 0.55 to 0.72) and 0.46 (95% CI, 0.33 to 0.57) in MZ, 0.42 (95% CI, 0.24 to 0.57) and 0.28 (95% CI, 0.08 to 0.47) in DZ pairs; heritability was 0.45 (95% CI, 0.12 to 0.71) and 0.42 (95% CI, 0.02 to 0.57). Age-adjusted partial correlation (APC) between Aixbra and body fat%, visceral fat, muscle%, BMI and metabolism indicated 0.14 ($p< 0.005$), -0.2195 ($p< 0.005$), -0.2619 ($p< 0.005$), -0.2026 ($p< 0.005$), -0.4282 ($p< 0.005$). APC between PWVao and body fat%, visceral fat, muscle%, BMI and metabolism indicated 0.1857 ($p< 0.005$), -0.0443 ($p=0.359$), -0.2054 ($p< 0.005$), 0.0586 ($p=0.221$).

Conclusion: Arterial stiffness parameters are heritable. A significant but moderate correlation between Aixbra and body consistency variables were identified. Lower correlation was estimated for PWVao.

Poster Session

A DESCRIPTIVE STUDY OF CARDIOVASCULAR RISK MANAGEMENT AND STATIN THERAPY IN BELGIAN AND NORWEGIAN GENERAL PRACTICE

Arne Svilaas[1], C. McGrath[2], H. van Campenhout[3], M. Grönsleth[3], X. Fournie[4], P. Kramarz[5], G. de Backer[6]

[1]Lipid Clinic, Department of Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [2]Medical Division, [3]European Outcomes Group, Pfizer Ltd, Tadworth, UK, [4]MAPI Group, Lyon, France, [5]European Centre for Disease Control, Pfizer Ltd, Stockholm, Sweden, [6]Dept. Public Health, Ghent University, Ghent, Belgium

Objectives: To describe the factors perceived by GPs in Belgium and Norway on their ability to evaluate and manage CVD risk including assessment, and to describe the actual care received by patients.

Methods: A cross-sectional survey of a randomly selected sample of GPs from Belgium and Norway was performed 2006-07. A subset of 20 consenting GPs per country abstracted data for a retrospective chart review of consecutively selected patients in their care. A descriptive analysis of each data set was performed.

Results: Clinical guidelines were highly valued by GPs, however less than half of them believed that reimbursement conditions were evidence based and useful. In Belgium, efficacy was the strongest factor driving choice of cholesterol lowering medicine wheras in Norway the strongest influence was reimbursement conditions. More than half of GPs in both countries always discussed CVD risk issues with their patients. More GPs reported setting cholesterol target values in Belgium compared to Norway. Statin treatment was initiated at a higher cholesterol level and target values attained more frequently in Norway than in Belgium.

Conclusions: CVD prevention in primary care can be improved through better detection of risk factors and setting simple treatment goals in cooperation with the patient. Constraints on physicians' time and flux in reimbursement conditions were perceived by GPs to place constraints on their options to treat patients with the most appropriate treatment for CVD risk. More research is needed into efficient ways of increasing the participation of GPs in epidemiological research aimed at increasing the quality.

Poster Session

ADIPOKINES IN MIDDLE-AGED WOMEN`S RISK PROFILE

Sybille Bergmann[1], K. Assig[2], F. Richter[3]

[1]TU Dresden, Universitätsklinikum Carl Gustav Carus, IKL, [2]TU Dresden, Universitätsklinikum, Dresden, [3]Krankenhaus Glauchau, Glauchau, Germany

Menopausal transition is followed by increasing cardiovascular risk and increasing prevalence of a Metabolic Syndrome. Beside age, fasting glucose, triglyzeride, blood pressure, HDL-C and LDL-C obesity (waist circumferrence, concentration of adipokines) may by a determinant of these risk scores. 314 female participants of the DRECAN_2005 survey (age >40 years; mean age 58 years; 147 postmenopausal) were included.

The prevalence of a MetS_IDF05 in the whole group was 20,7% (postmenopausal subgroup 21,8%. Lower level of adiponectin and higher concentration of leptin among women with MetS were accompanied by higher concentration of CRP and leukocyte count.

The ten- year- risk of a cardiovascular event (PROCAM calculator) was higher in women with MetS_IDF05 (11,9±5,1% vs. 7,1±5,5%).

In the whole group but not among postmenopausal women the concentration of adiponectin was age-dependent. Both adipokines were correlated with BMI: (A) r=-0,362; p< 0,001); (L) r=0,662; p< 0,001). After adjustment for age and BMI the positive correlation of leptin and the PROCAM score disappeared whereas the weak inverse correlation of adiponectin became stronger in the whole group (r=-0,157 ; p< 0,05) and was unchanged among postmenopausal women (r=-0,277; p< 0,001). Subanalysis of factors contributing to the risk scores showed an inverse correlation of adiponectin with HDL-C , ApoAI/ApoB-ratio, HOMA-IR in the whole group and additionally with TG among postmenopausal women. Leptin was associated positively with TG, HOMA-IR ,waist circumferrence in the whole group and additionally inversely with HDL-C in the postmenopausal subgroup.

Among middle-aged women, adiponectin is more strongly related to the cardiovascular risk than leptin.

Poster Session

ROLE OF THE ADAPTOR PROTEIN PID1 IN LRP1 MEDIATED ENDOCYTOSIS

Britta Hoffzimmer[1], P. Gordts[2], H. Schmale[1], J. Heeren[1]

[1]IBMII: Molecular Cell Biology, University Hospital Hamburg Eppendorf, Hamburg, Germany, [2]Katholieke Universiteit Leuven, Leuven, Belgium

Objective: Abnormalities in lipoprotein metabolism are associated with the development of atherosclerosis and the metabolic syndrome. The uptake and intracellular processing of lipoproteins directly influence the plasma lipoprotein levels and both processes are mediated by hepatic lipoprotein receptors such as the LDL-receptor and the LDL-receptor-related-protein1 (LRP1). Next to triglyceride-rich lipoproteins, LRP1 can also bind to various other ligands indicating that different effects are mediated by specific LRP1 adaptor proteins. The aim of this project is to investigate how the newly identified LRP1 adaptor phosphotyrosine-interaction-domain-containing-protein1 (PID1) modulates LRP1 function with the focus on internalisation and endosomal trafficking.

Methods: Interaction of LRP1 with PID1 was analysed by pull-down experiments after mutation of both NPXY motifs of LRP1 *in vitro* and by co-immunoprecipitation *in vivo*. Overexpression and shRNA-mediated knock-down of PID1 was performed by lentiviral transfer into human hepatoma cells HUH7. The uptake of the LRP1-ligand $I^{125}\alpha_2$-macroglobulin was investigated after overexpresssion and knock-down of PID1 in HUH7 cells.

Results: Here we show that PID1 binds specifically to the distal NPXY motif within the cytoplasmic tail of LRP1. Tyrosine-phosphorylation of the distal NPXY motif by SRC-kinase inhibits the interaction of LRP1 with PID1. Knock-down of PID1 in HUH7 cells decreases the uptake of α_2-macroglobulin while overexpression of PID1 has the opposite effect. These data suggest that the LRP1-PID1-interaction alters receptor mediated endocytosis indicating the functional importance of PID1 for LRP1 function.

Conclusion: PID1 interacts with the distal NPXY motif of the LRP1 intracellular domain and regulates LRP1 mediated endocytosis in human hepatoma cells.

Poster Session

GLYCATED LDL STIMULTE FOAM CELL FORMATION *IN VITRO* AND LDL GLYCATION IS OPPOSED BY PARAOXONASE-RICH HDL

N. Younis[1,2], **Handrean Soran**[1], R. Sharma[1], P. Pemberton[3], V. Charlton-Menys[1], M. Elseweidy[2], P. Durrington[1]

[1]The University of Manchester, Manchester, UK, [2]Zagazig University, Faculty of Pharmacy, Biochemistry Department, Zagazig, Egypt, [3]Clinical Research Department, Manchester Royal Infirmary, Manchester, UK

Introduction: Oxidative modification has attracted most interest, but glycated LDL present in the circulation at much higher concentration than oxidized LDL. We have recently reported that LDL glycation occurs predominantly in SD-LDL. Foam cells, central to atherogenesis, do not form when monocyte-macrophages are exposed to unmodified LDL.

Method: To show the atherogenicity of glycated LDL we used THP-1 derived macrophages to examine the ability of glycated LDL to induce cholesterol uptake and foam cell formation. The macrophages were incubated with the glycated and non-glycated LDL for 2 days and stained with oil red O. We have conducted experiments to determine if the cholesterol content of THP-1 cells incubated with LDL in the presence of HDL is decreased. We assessed the effect of HDL on LDL glycation. We incubated LDL and glucose *in vitro* in the presence of HDL, HDL from patients with low PON1 activity and avian HDL (lacking PON1).

Results: Cellular lysates from similarly incubated cells with glycated and non-glycated LDL showed significantly higher cholesterol content from glycated LDL-incubated cells than the non-glycLDL-incubated cells. Incubating LDL with glucose *in vitro* revealed that SD-LDL was more susceptible to glycation than more buoyant LDL ($P < 0.01$) and the glycation was decreased by HDL ($P < 0.01$), but not by HDL from patients with low paraoxonase 1 activity (PON1) or by avian HDL.

Conclusion: We conclude that glycation of apoB is an important atherogenic modification that triggers foam cell formation. HDL can protect LDL against glycation by a mechanism which may involve PON1.

Poster Session

ROLE OF FARNESOID X RECEPTOR AGONISTS IN THE *IN VIVO* AND *IN VITRO* EXPRESSION OF APOLIPOPROTEIN(A)

Indumathi Chennamsetty, G. Kostner

Molecular Biology and Biochemistry, Medical University Graz, Graz, Austria

Objectives: High plasma concentrations of lipoprotein(a) (Lp(a)) have been implicated as a major independent risk factor for atherosclerosis. In humans, Lp(a) is mostly expressed in the liver. Plasma Lp(a) concentrations are mostly determined by the synthesis rate of its protein part apo(a). Apo(a) is coded by a large gene for which considerable controversy exist concerning factors that regulate its expression.

Methods: The influence of bile acids and synthetic FXR agonists was studied *in vivo* by using FXR wt/apo(a) and FXR deficient/apo(a) mice and *in vitro* by using primary hepatocytes from apo(a) transgenic mice. Wild-type and FXR-deficient mice were divided into 2 groups and one group received a standard rodent chow diet, where as the other group received the same diet containing 0.2% (wt/wt) of cholic acid for 5 days. Mice were bled regularly to check the plasma apo(a) levels and were sacrificed on day 5 to collect liver,ileum samples for RNA, protein analysis and for histology.

Results: In wild-type mice, feeding of FXR agonist cholic acid (0.2%) reduced plasma apo(a) levels and down-regulated apo(a) expression significantly at both mRNA and protein levels. Treatment of primary hepatocytes from apo(a) transgenic mice with cholic acid or GW 4064 resulted in a significant decrease in apo(a) expression.

Conclusion: We identified bile acid and synthetic agonists of FXR as negative regulators of apo(a) expression.

Poster Session

ADIPOSE TRIGLYCERIDE LIPASE DEFICIENCY RESULTS IN MACROPHAGE APOPTOSIS

Elma Aflaki[1], R. Malli[1], B. Radovic[1], T. Eisenberg[2], J. Ring[2], P.G. Chandak[1], W. Graier[1], F. Madeo[2], D. Kratky[1]

[1]Institute of Molecular Biology and Biochemistry, Medical University of Graz, [2]Institute of Molecular Biosciences, University of Graz, Graz, Austria

Macrophage death is a prominent feature of atherosclerotic lesions. *In vivo* studies have shown that intracellular accumulation of free cholesterol leads to macrophage apoptosis. We found that adipose triglyceride lipase (ATGL), the major enzyme responsible for triglyceride hydrolysis in many tissues, is expressed in mouse peritoneal macrophages (MPM). Since absence of ATGL causes triglyceride accumulation we used MPM of ATGL-deficient mice to examine the effect of triglyceride accumulation on macrophage cell death which has not been studied in detail so far.

Annexin V/propidium iodine staining revealed that, ATGL deficiency in MPM results in apoptosis. Expression of anti-apoptotic Bcl-XL and MCL1 is nearly abolished, whereas expression of pro-apoptotic Bax is markedly increased in ATGL-deficient MPM. Reduced mitochondrial membrane potential and elevated reactive oxygen species production and cytochrome C release indicate mitochondrial dysfunction. Furthermore, caspase-3 activation leads to poly (ADP-ribose) polymerase cleavage. Moreover, ATGL deficiency in macrophages causes caspase-12 activity and increased CHOP expression, a pro-apoptotic C/EBP homologous protein that is induced by endoplasmic reticulum (ER) stress.

We conclude that ATGL has a pivotal role in macrophages survival and its absence, which results in massive triglyceride accumulation, leads to apoptotic cell death due to mitochondrial dysfunction and ER stress.

Poster Session

SAA ACTIVATES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR Γ THROUGH EXTRACELLULAR-REGULATED KINASE 1/2 AND COX-2 EXPRESSION IN HEPATOCYTES

Hongzhe Li[1], Y. Zhao[2], S. Zhou[1], C.-K. Heng[1]

[1]NUS, Singapore, Singapore, [2]Advanced Institute of NBIC Integrated Drug Discovery and Development, East China Normal University, Shanghai, PR China, Shang hai, China

Objective: Serum amyloid A (SAA) is an acute phase protein whose expression increases markedly during inflammation. It is an attractive therapeutic target for the treatment of atherosclerosis. The objective of this study was to investigate the effects of SAA on peroxisome proliferator-activated receptor γ (PPARγ) activation and its downstream effects in human hepatocytes (HepG2).

Methods: Quantitative real-time PCR, Western-blot, PPARγ transcriptional activity assay, 15d-PGJ_2 EIA assay and cholesterol efflux assay were employed in this study to investigate the effects and mechanisms of SAA on PPARγ activation.

Results: We demonstrated that SAA could induce the PPARγ transcriptional activity by more than two folds and up-regulate the expression of ABCA1 and ABCG1 by more than two folds. Preincubation of HepG2 cells with SAA enhanced cholesterol efflux to high density lipoprotein (HDL) and apoA-I by 23% and 100%, respectively. In addition, SAA increased intracellular 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), which is a potent natural ligand for PPARγ, by more than 3 folds. Pretreatment with ERK1/2 inhibitor and COX-2 inhibitor could partially inhibit the SAA effects on PPARγ and its target genes.

Conclusions: Our data suggested that SAA activated PPARγ through ERK1/2 dependent COX-2 expression and subsequently enhanced the cholesterol efflux by inducing ABCA1 and ABCG1. Overall, our study has established, for the first time, a relationship between SAA and PPARγ. Additionally, the data from our study has also provided new insights into the role of SAA in cholesterol efflux and the development of atherosclerosis.

Poster Session

ENDOGENOUS APOLIPOPROTEIN E REGULATES ADIPOGENIC DIFFERENTIATION

Dorothee Lasrich, A. Bartelt, A. Laatsch, U. Beisiegel, J. Heeren

Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

Objectives: Besides other organs apolipoprotein E (apoE) is highly expressed in adipose tissue but its role in adipocytes is poorly understood. In this study we investigate the function of endogenous apoE in a human adipocyte cell line and primary murine adipocytes.

Methods: Human adipocytes were stably transduced with siRNA directed against apoE using a lenti-viral approach. Primary murine adipocytes were isolated from wild-type and apoE knock-out mice. Cells were differentiated into adipocytes following the standard protocol.[1] Lipid accumulation was monitored by oil-red-o staining and expression of adipocyte marker genes was analysed using quantitative real-time PCR and Western blot.

Results: ApoE knock-down cells accumulated markedly less triglycerides compared to control cells. This is in line with a dramatically decreased expression of adipocyte markers such as Adiponectin, FABP4 and PPARγ, especially PPARγ2. This disturbed adipocyte differentiation was also observed in primary murine apoE-deficient adipocytes. Supplementation experiments with recombinant apoE, conditioned media from apoE3-overexpressing adipocytes and apoE-containing VLDL could not restore the adipocyte marker expression but led to dramatic increase of triglyceride accumulation in those cells.

Conclusion: While exogenous apoE seems to be important for lipoprotein metabolism in adipocytes, solely the endogenous apoE is able to restore adipogenic differentiation. These data suggest that endogenous adipocyte-derived apoE is important for the differentiation in a way that is independent from its effect on triglyceride uptake.

Reference: 1. Prawitt J *et al.,* Exp Cell Res, **2008** Feb;314(4):814-24

Poster Session

PROTEIN KINASES ARE KEY REGULATOR OF OX-LDL INDUCED IL-1B SECRETION

Rajiv Tiwari[1], V. Singh[1], A. Verma[2], M. Dikshit[1], M. Barthwal[1]

[1]CVS Unit, Pharmacology, Central Drug Research Institute, [2]Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow, India

Atherosclerosis involves series of events that are accompanied with hyperlipidemia and inflammation. Ox-LDL is known to induce inflammatory response during atherosclerosis progression and IL-1β is one of the important proatherogenic cytokine involved in such a phenomenon. The present study was undertaken to investigate the role of protein kinases in oxidized LDL (Ox-LDL) induced IL-1β secretion. A time dependent increase in IL-1β secretion was observed in Ox-LDL (40µg/ml) treated human monocytic THP-1 cells. Expression of TLR-2 and CD-36 were also increased after Ox-LDL treatment. Pretreatment with inhibitors of JNK(10µM), PKC-δ (Rottlerin,2µM), Src-kinase inhibitor (PP2,100nM) or IL-1receptor antagonist(60µM) significantly reduced Ox-LDL induced IL-1β secretion by ~81%,~38%, ~72% and ~80% respectively. Pretreatment with p38MAPK inhibitor (SB202190, 200µM) or PI3K inhibitor (LY294002, 100µM) had no significant effect on Ox-LDL induced IL-1β secretion. Although Ox-LDL increased the AP-1 and NF-kB activity, both small molecule (1µM) and peptide inhibitor (SN50, 20µM) of NF-kB failed to prevent Ox-LDL induced IL-1 β secretion indicating role of NF-kB independent pathway. Since Ox-LDL induced JNK phosphorylation was inhibited by rottlerin it indicates its downstream position. Results from the present study thus indicate that PKC-δ, JNK, Src kinases play an important role in IL-1β secretion.

Poster Session

ACTIVATION OF UNFOLDED PROTEIN RESPONSE SIGNALING MEDIATES APOLIPOPROTEINE-DEFICIENT LIPOPROTEIN-INDUCED FOAM CELL FORMATION

Hong Yang, Y. Zhao, Z. Guo

Meharry Medical College, Nashville, TN, USA

Transformaion of macrophages into foam cells is a early stage of atherogenesis. Apolipoprotein (Apo) E-deficient, ApoB48-containing (E^-/B48) lipoprotein-induced foam cell formation has been shown to be associated with an increased phosphorylation of eukaryotic initiation factor-2α (eIF-2α), a hallmark feature of unfolded protein response. The objective of this study is to examine the impact of a nonphosphorylatable eIF-2α mutant on E^-/B48 lipoprotein-induced changes in gene expression and foam cell formation. We observed that incubation of mouse peritoneal macrophages (MPMs) with E^-/B48 lipoproteins reduced the protein levels of cholesterol transporters and lysosomal hydrolases, and enhanced eIF-2α phosphorylation. Transfection of MPMs with an eIF-2α mutant, in which serine 51 was replaced with alanine, alleviated the suppressive effect of E^-/B48 lipoproteins on lysosomal hydrolase and cholesterol transporter expression, increased macrophage degradation of E^-/B48 lipoproteins, enhanced cholesterol efflux, and suppressed foam cell formation. These observations suggest that induction of eIF-2α phosphorylation is a mechanism by which ApoE-deficient lipoproteins transform macrophages into foam cells.

This study is supported by NIH SC1HL101431 (H.Y.)

Poster Session

CARRIERS OF LCAT GENE MUTATIONS HAVE INCREASED ATHEROSCLEROSIS: A 3.0 TESLA MRI STUDY

Adriaan G. Holleboom[1], R. Duivenvoorden[1], B. van den Bogaard[1], E. de Groot[1], A.J. Nederveen[2], J.S. Lameris[3], J.J.P. Kastelein[1], E.S.G. Stroes[1], J.A. Kuivenhoven[1]

[1]Vascular Medicine, [2]Medical Physics, [3]Radiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Background: Heterozygous carriers of mutations in lecithin:cholesterol acyltransferase (LCAT) present with an atherogenic lipid profile characterized by half normal plasma levels of high-density lipoprotein cholesterol (HDL-c) and moderately elevated triglyceride levels but the effects on atherosclerosis are not clear.

Methods: 3.0 Tesla MRI scans were run to acquire Axial T1-weighted TSE images of the carotid arteries of 41 heterozygous carriers of LCAT mutations (mean age 43 years, SD 14) and 54 unaffected age and gender matched controls (mean age 41 years, SD 15). Participants referred to the clinic for cardiovascular disease were excluded. Carotid Mean Wall Area (MWA, mm^2) and Normalized Wall Index (NWI), defined as the ratio between the wall and outer wall area, were quantified.

Results: Statistically significant differences in lipid levels in carriers and controls were: HDL-c 0.8 (SD 0.3) versus 1.5 (SD 0.4) mmol/l ($p< 0.001$), triglycerides 1.8 (SD 1.7) versus 1.1 (SD 1.3) mmol/l ($p=0.02$). Mutations in LCAT were shown to be functional. In the carriers, MWA was 18.2 (SD 0.9) mm^2, versus 14.2 (SD 0.4) mm^2 in controls ($p=0.01$). NWI was 0.34 (SD 0.07) versus 0.31 (SD 0.05, $p=0.01$). In multivariate analysis the heterozygosity for mutations in LCAT was a predictor of MWA ($p=0.003$) and NWI ($p=0.007$), independent of age, sex and low-density lipoprotein cholesterol (LDL-c).

Conclusion: Carriers of LCAT mutations have increased vessel wall thickness of the carotid arteries, indicative of increased cardiovascular risk.

Poster Session

MOUSE GENE EXPRESSION NETWORKS INDICATE MITOCHONDRIAL DYSFUNCTION CAUSES METABOLIC INFLAMMATION IN OBESITY: VALIDATION BY HUMAN GENE EXPRESSION AND SNP ANALYSIS

James Scott, X. Li

Medicine, Imperial College London, London, UK

Insulin resistance is causally linked to macrophage-driven “metabolic inflammation” in white adipose tissue (WAT) from the obese. We used gene expression, co-variation networks to identify core functional perturbations associated with adiposity, inflammation and insulin resistance in WAT from diet induced obese mice . Principal component analysis showed differences between fat-fed and control mice. Hierarchical cluster, Ingenuity Pathway and gene set enrichment analysis showed high-fat feeding rapidly produces florid gene expression changes in WAT, many of which are also displayed in a more subdued way in chronic obesity (p-value < 10^{-104}). We developed gene expression co-variation networks and sub-networks, which includes data from multiple models. The most strongly co-varying genes are mitochondrial (p-value < 10^{-121}); with sub-networks corresponding to oxidative phosphorylation, lipogenesis and β-oxidation (p-value < 10^{-55}). Distinct inflammatory sub-networks were also discovered (p-values < 10^{-5}). Genes associated with metabolic phenotypes in GWASs were identified within the metabolic sub-networks, and included *Mlxipl, Pank1*, *Mtch2, Ide* and *Jazf1*, respectively associated with hypertriglyceridaemia, insulin levels, obesity and T2D. We evaluated gene expression data from human WAT, and also found suppression of oxidative phosphorylation and β-oxidation (P value < 10^{-12}) and activation of inflammation (P value < 10^{-4}). We tested human SNPs in genes corresponding to those in the mouse networks for genome-wide association with metabolic phenotypes and these are undergoing replication. We conclude that mitochondrial dysfunction in response to high-fat feeding and in obesity favours obesity and inflammation in WAT.

Poster Session

TWO NOVEL GENETIC LOCI FOR HIGH AND LOW PLASMA HDL-C LEVELS IN 2 LARGE FAMILIES FROM DUTCH ANCESTRY

Mohammad Mahdi Motazacker[1], I. Tietjen[2], M. Hayden[2], J.P.P. Kastelein[1], E.S.G. Stroes[1], G.K. Hovingh[1], J.A. Kuivenhoven[1]

[1]Academic Medical Center Amsterdam, Amsterdam, The Netherlands, [2]Xenon Pharmaceuticals Inc., Burnaby, BC, Canada

Plasma high-density lipoprotein-cholesterol (HDL-c) levels are inversely correlated with cardiovascular disease (CVD). In this light, therapeutic targeting of HDL is regarded as an interesting strategy to reduce atherosclerosis. Plasma HDL-c level is a complex trait and its genetic component has recently been studied by many genome-wide association studies (GWAS). Interestingly, only 7 loci have thus far been associated with this trait which can explain a mere 5.2% of the heritability. This may indicate that rare variants with large or intermediate effect on the phenotype may account for a large portion of this unexplained heritability.

In search for rare variants determining plasma HDL-c levels, we have performed linkage analysis studies in 2 large families with an autosomal dominant pattern of high HDL-c (>95th%) or low HDL-c (< 5th%) phenotypes for age and gender (mutations in major candidate genes were excluded). We identified a novel locus for high plasma HDL-c levels (4.5 MB interval with multipoint parametric LOD score 3.4) and a novel locus for low plasma HDL-c levels (8 MB interval with multipoint parametric LOD score 4). To identify underlying genetic defects in these linkage intervals, we have designed capture arrays for targeted enrichment of the coding sequence and regulatory elements of the genes within each interval which will be processed by massive parallel sequencing. Our Pilot data indicates that many more ´HDL genes´ are waiting to be discovered and that family studies are likely to remain an important tool for progress in this field.

Poster Session

CARRIERS OF LCAT GENE MUTATIONS HAVE HIGH DENSITY LIPOPROTEIN WITH DECREASED ANTI-OXIDATIVE CAPACITY

Adriaan G. Holleboom[1], G. Daniel[2], G.K. Hovingh[1], A.W. Schimmel[1], J.N. van Miert[1], J.J.P. Kastelein[1], E.S.G. Stroes[1], J.A. Kuivenhoven[1], A. Chroni[2]

[1]Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands, [2]Institute of Biology, National Center for Scientific Research 'Demokritos', Athens, Greece

Background: In *vitro* and animal studies have shown that lecithin:cholesterol acyltransferase (LCAT), an enzyme involved in high density lipoprotein (HDL) metabolism, has anti-oxidant properties. It is unclear, however, whether HDL of carriers of LCAT gene mutations has decreased anti-oxidative capacity.

Methods: HDL of 69 heterozygous carriers and 62 unaffected family members was isolated from plasma and incubated in equal concentrations with human oxidized LDL. The capacity of HDL to inactivate the oxidized phospholipids of LDL was measured in the presence of 2,7 dichlorofluorescin (DCFH) that reacts with lipid peroxidation products.

Results: Statistically significant differences in lipid levels of heterozygous carriers and controls were: HDL-c 0.8 (SD 0.3) versus 1.4 (SD 0.55) mmol/l ($p < 0.0001$), apolipoprotein A-I 159 (SD 33) versus 127 (SD 24) mg/dl ($p < 0.0001$) and triglycerides (median (interquartile range)) 1.6 (0.9-2.3) versus 1.0 (0.69-1.21) mmol/l (p after log-transformation < 0.05).

The anti-oxidative capacity of HDL was significantly decreased in the carriers compared to controls: fluorescence intensity in carriers was $3,4 * 10^4$ arbritrary units (AU) versus $2,3 * 10^4$ AU in controls ($p < 0.0001$). This association was independent of age and gender. When 4 patients homozygous for a functional LCAT mutation were included in the analysis, the decrease in anti-oxidative capacity was found to be gene dose-dependent (p for ANOVA < 0.0001).

Conclusion: This first report of the anti-oxidative properties of LCAT in humans suggests that LCAT has a physiologically relevant anti-oxidative function in human lipoprotein metabolism, which is abolished in LCAT deficiency.

Poster Session

THE LEPTIN RECEPTOR GLN223ARG POLYMORPHISM MEDIATES THE POSTPRANDIAL LIPAEMIC RESPONSE IN ADULT MALES

Kim Jackson[1], E. Olano-Martin[1], E. Abraham[1], R. Gill[2], A. Valdes[3], F. Tang[1], J. Lovegrove[1], C. Williams[1], A. Minihane[1]

[1]University of Reading, Reading, UK, [2]Ipsogen Inc, Stamford, CT, USA, [3]King's College London, London, UK

Background and aims: An exaggerated postprandial triacylglycerol (TAG) response is an important determinant of cardiovascular disease risk. The main genetic modulators of TAG metabolism include variants of the apolipoprotein (apo)E, apoCIII, apoAV and lipoprotein lipase genes. Our aim was to examine the impact of the leptin receptor (LEPR) Gln223Arg polymorphism on postprandial lipaemia in healthy individuals.

Methods: Males (n=122) and females (n=109) underwent a sequential meal postprandial investigation, in which blood samples were taken at regular intervals after a test breakfast and lunch given at 0 and 330 min respectively.

Results: Fasting total- and low density lipoprotein cholesterol were 9% lower in the ArgArg compared with the GlnArg genotype ($P< 0.04$), whereas fasting TAG was 27% lower in the ArgArg compared with the GlnGln group ($P< 0.02$). The magnitude of the postprandial TAG response was also significantly lower in the ArgArg compared with the GlnArg and GlnGln genotypes, with a 26% ($P=0.010$) and 31% ($P=0.023$) lower area under the curve (AUC) and incremental AUC (IAUC) in the ArgArg individuals. Gender*genotype interactions were evident for the fasting TAG, TAG AUC and IAUC ($P< 0.05$), with the genotype effect only evident in males. Regression analysis indicated that the LEPR genotype and genotype*gender interactions were independent predictors of the TAG AUC, accounting for 6.3% of the variance.

Conclusion: We report for the first time that the common LEPR Gln223Arg genotype is an important predictor of the postprandial TAG response in males. The mechanistic basis of these associations remains to be evaluated.

Poster Session

LIPID DROPLETS IN HUMAN MACROPHAGES RECRUIT CYTOSOLIC PHOSPHOLIPASE A_2 AND PROMOTE SECRETION OF INFLAMMATORY MEDIATORS

Clara De Pascale, P. Bostrom, Y. Wickström, C. Mogensen, L. Mattsson Hultén, R. Perkins, M. Ståhlman, O. Wiklund, S.-O. Olofsson, J. Borén

Sahlgrenska Center for Cardiovascular and Metabolic Research, Wallenberg Laboratory, University of Gothenburg, Sahlgrenska University Hospital, Göteborg, Sweden

Foam cells, inflammation and hypoxia characterize early atherosclerotic lesions. Previous studies have shown that exposure of human macrophages to hypoxia and oleic acid results in the accumulation of neutral lipids (triglycerides and cholesteryl esters) in cytosolic droplets. Foam cell formation is a lipid disorder but it is also associated with inflammatory events. In this study, we investigated the relationship between lipid droplet formation and the induction of inflammation in human macrophages. Incubation of macrophages with oleic acid and in hypoxic conditions resulted in increased secretion of the eicosanoids leukotriene B_4 (LTB_4) and prostacyclin (PGI_2) and the chemokines RANTES and interferon-inducible protein 10 (IP-10). Both treatments also promoted the translocation of cytosolic phospholipase A_2 ($cPLA_2$), an enzyme involved in the eicosanoid production, to the lipid droplet fraction. Transfection of hypoxic macrophages with SNAP23 siRNA, which inhibits lipid droplet fusion and thus increases the lipid droplet surface area, induced substantial increases in $cPLA_2$ levels on lipid droplets and in the secretion of inflammatory mediators. Chemokine secretion was inhibited by $cPLA_2$ inhibitors and LTB_4 receptor antagonists, suggesting that this is mediated by increased production of eicosanoids. In conclusion, our data show that $cPLA_2$ may be a direct link between cellular metabolism and inflammatory responses by mediating the formation of eicosanoids and the secretion of chemokines. These inflammatory events may be critical in pathologies such as type 2 diabetes and atherosclerosis where high levels of fatty acids are observed.

Poster Session

COMPOSITE DEFICIENCY OF THE LIPOLYTIC COMPLEX IN PREGNANCY-INDUCED MAJOR HYPERTRIGLYCERIDEMIA

Pascale Benlian[1], B. Donadille[2], C. Bouché[3], C. Vincent Dejean[4], R. Valero[5], F. Dufernez[6], V. Sapin[7], F. Paye[8], P. Bouchard[2]

[1]Biochemistry and Molecular Biology, Université Pierre et Marie Curie, [2]Endocrinology, Hôpital Saint Antoine, APHP, [3]Endocrinology, Hôpital Saint Louis APHP, Paris, [4]Endocrinology, Hôpital Pasteur, Chartres, [5]Endocrinology, Hôpital Timone, APHM, Marseille, [6]Biochemistry and Molecular Biology, Hôpital Saint Antoine, APHP, [7]Biochemistry and Molecular Biology, CHU Clermont Ferrand, [8]Digestive Surgery, Hôpital Saint Antoine, APHP, Paris, France

Background: Moderate hypertriglyceridemia (HTG) is common at the last trimester of normal pregnancy, as the result of high oestrogen secretion. Although exceptional, major HTG may have life-threatening consequences for the mother and child, through a risk of acute pancreatitis before delivery or in post partum. Chylomicronemia may occur as the result of genetic variations of the endothelial lipolytic complex, in metabolic conditions challenging lipoprotein lipase (LPL) activity, however no extensive analysis is usually reported beyond one disease-locus.

Aims: To explore major genes regulating LPL activity in pregnant women experiencing major HTG.

Patients and methods: Women with 12h fasting plasma TG>100 mg/dL during the last trimester of pregnancy or in early post-partum agreed for a genetic testing and a measure of post-heparin LPL activity, measured by spectrofluorimetry (Progen). APOA5, APOC2, APOE and LPL were sequenced (ABI 3130, Applera) and files interpreted with Gensearch (Phenosystems). LPL gene rearrangements were analysed by MLPA (MRC-Holland).

Results: Among 10 women aged 23-38, whom thought themselves normolipidemic, 9 had major HTG (106-1,580 mg/dL) during pregnancy; 1 in the 1st Mo. post-partum. A history of acute pancreatitis was present in 5, and of recurrent abdominal pain in 1. Fasting plasma HDL was low (24±8 mg/dL). Plasma LPL activity was low to undetectable in 6, and normal in 2. Eight were mutation carriers; 6 were composite carriers of mutations or common variations at the LPL, APOE, or APOA5 loci.

Conclusion: Composite defective genotypes of the lipolytic complex are prevalent causes of pregnancy-induced HTG, in otherwise majorly normolipidemic women.

Poster Session

INTERACTION OF PARAOXONASE 1 WITH LIPID RAFT MICRO-DOMAINS FOR THE ENHANCEMENT OF CHOLESTEROL EFFLUX

Hicham Berrougui, O. Helal, T. Said, A. Khalil

Research Centre on Aging, Geriatric Service, Department of Medicine, University of Sherbrooke, Sherbrooke, QC, Canada

Introduction: It is now well established that the anti-atherogenic properties of HDL are related to their capacity to protect LDL against lipid-peroxidation and to promote reverse cholesterol transport. We have previously demonstrated that antioxidant activity of HDL decreases during ageing, in part, as a consequence of the alteration of paraoxonase 1 (PON1). However, the role of PON1 in the modulation of cholesterol efflux process was until now poorly understood.

Objective: The aim of this work was to investigate the role of PON1 in HDL-mediated cholesterol efflux process.

Methods and results: Our results show that purified human PON1 promotes in a dose dependent manner HDL-mediated cholesterol efflux in J774 as well as in THP-1 macrophages. Furthermore, over expression of ABCA1-transporter by J774 cells induces in a significant increase of PON1 stimulating HDL-mediated cholesterol efflux. Moreover, PON1 alone was able to remove free cholesterol excess from macrophages. This effect was more pronounced in the lipid raft fractions than in the rest of cell compartment. Western blot analysis, upon short incubation of PON1 with macrophages, shows that PON1 enhances ABCA1 expression. These results suggest that PON1 enhances cholesterol efflux by interacting with lipid raft. Functional studies demonstrated that alteration of PON1 SH group affects its capacity to mediate cholesterol efflux.

Conclusion: Our data show that PON1 plays a key role in the cholesterol efflux processes via an ABCA1 dependent pathway, and that this property can be related to the free -SH groups.

Poster Session

OXLDL STIMULATES LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 EXPRESSION IN THP-1 MONOCYTES VIA PI3K AND P38 MAPK PATHWAYS

Yiping Wang, W. Wang, Y. Leng, J. Shen

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica,Chinese Academy of Sciences, Shanghai, China

Aims: Lipoprotein-associated phospholipase A_2 (lp-PLA_2) has been detected in human and rabbit atherosclerotic lesions, where it co-localizes with its substrate, oxidized LDL (oxLDL). Here, we investigated whether oxLDL may exert a regulatory effect on lp-PLA_2 expression.

Methods and results: Using human monocytic THP-1 cells as a model system, we found that oxLDL up-regulated the expression of lp-PLA_2 while another substrate of the enzyme, platelet activating factor (PAF), had no such effect. The up-regulatory effect of oxLDL could be conferred by its oxidized phospholipids (oxPCs, the exact substrates of lp-PLA_2), but not their hydrolyzed products, lysophosphatidylcholines (lysoPCs). OxLDL induced the activation of p38 mitogen-activating protein kinase (MAPK) through phosphatidylinositol 3-kinase (PI3K). Inhibition of either PI3K or p38 MAPK completely blocked oxLDL-induced lp-PLA_2 expression. In addition, inhibition of lp-PLA_2 activity in the conditioned medium significantly decreased lipid accumulation in macrophages as detected by oil red staining.

Conclusions: The present study shows that oxLDL, and more specifically its unhydrolyzed oxidized phospholipids, can up-regulate lp-PLA_2 expression in monocytes through the PI3K and p38 MAPK pathway. In turn, lp-PLA_2 promotes lipoprotein uptake in macrophages. Our results uncover a new link between oxLDL and lp-PLA_2, and may provide insight into this interaction in the context of atherosclerosis.

Poster Session

SCAVENGER RECEPTOR CD36 MEDIATES HDL SELECTIVE CHOLESTERYL ESTER UPTAKE AND HDL PARTICLE INTERNALISATION IN VITRO

May Brundert, P. Groitl, J. Heeren, T. Dobner, F. Rinninger

University Hamburg, Hamburg, Germany

Scavenger receptor CD36-deficient mice (Knockout) may have an increase in HDL cholesterol in plasma compared to wildtype. CD36 binds HDL in vitro. However, the function of CD36 in HDL metabolism is controversial at present.

The role of CD36 for HDL holo-particle internalisation and for HDL selective cholesteryl ester (CE) uptake was investigated in H1299 cells. Murine CD36 was expressed with a recombinant adenovirus (Ad-mCD36) which mediates the expression of this receptor. Murine HDL was labeled with 125-I (protein) and [3H] (CE).

In H1299 incubated in medium alone (mock) or in the presence of Ad-GFP (control), no CD36 was detected in immunoblots. However, Ad-mCD36 induced a virus dose-dependent expression of CD36 as detected in immunoblots. H1299 cells incubated (37°C) in medium containing Ad-mCD36 or Ad-GFP. Thereafter followed an incubation in the presence of radiolabeled HDL (40 µg protein/ml). Ad-mCD36 induced a dose-dependent increase in internalisation of HDL-associated 125-I compared to Ad-GFP; at MOI 200, 125-I uptake increased significantly by 447% (Ad-GFP 100%). Thus CD36 induces an increase in HDL particle uptake. Besides, Ad-mCD36 (MOI 200) stimulates uptake of [3H] from HDL significantly by 239%, suggesting an increase in cellular HDL CE uptake. Selective CE uptake from HDL ([3H] - 125-I) increased significantly by 204%. and also this stimulation was dependent on the dose of Ad-mCD36.

In summary, in vitro CD36 promotes the internalisation of HDL holo-particles and stimulates selective CE uptake from HDL. A role of scavenger receptor CD36 for HDL metabolism is suggested.

Poster Session

TIP47 AFFECTS TRIGLYCERIDE CONTENT IN MACROPHAGE FOAM CELLS

Insa Buers[1], O. Hofnagel[2], H. Robenek[1]

[1]Leibniz-Institute for Arteriosclerosis Research, Muenster, [2]Max-Planck-Institute of Molecular Physiology, Dortmund, Germany

Background: A characteristic of early atherosclerotic lesions is the accumulation of foam cells with massive amounts of lipid droplets (LD). A LD consists of a core of neutral lipids surrounded by a monolayer of phospholipids which contains a cell-type specific composition of LD associated proteins, e.g. adipophilin and TIP47. They are implicated in lipid droplet formation but the precise function of TIP47 is poorly defined. The aim of our study was to investigate how TIP47 influences macrophage (MΦ) LD formation and composition.

Material and results: Freeze-fracture cytochemistry demonstrates that TIP47 is present in the plasma membrane of THP-1 MΦ and is aggregated into clusters when the cells are incubated with oleate. Confocal microscopy reveals that suppression of adipophilin leads to migration of TIP47 from the cytoplasm to the lipid droplet surface, whereas suppression of TIP47 does not influence adipophilin distribution. Cholesterol and triglyceride levels where determined using the GPO/PAP and CHOD/PAP method. Suppression of TIP47 decreased triglyceride levels, whereas the increase of TIP47 levels by expression of EGFP-TIP47 showed the opposite effect.

Conclusions: TIP47 is redistributed from the cytosol to LD in MΦ lacking adipophilin. Due to the high sequence homology we propose comparable cholesterol and triglyceride binding properties of TIP47 and adipophilin. However, our results showed that the TIP47 protein levels directly correlate with triglyceride levels. We propose that TIP47 may act as a lipid carrier protein and in this way participates in conversion of MΦ into foam cells.

Poster Session

NON-CHOLESTEROL STEROLS IN COMMON HYPERCHOLESTEROLEMIA AND FAMILIAL COMBINED HYPERLIPEMIA

Graziana Lupattelli, M. Pirro, D. Siepi, M.R. Mannarino, A.R. Roscini, G. Vaudo, G. Schillaci, E. Mannarino

Dept of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy

Objective: Human plasma contains amounts of lathosterol, a marker of cholesterol biosynthesis, and plant sterols, sitosterol and campesterol, surrogate markers for cholesterol absorption. We investigated the behaviour of these sterols in subjects with primary hyperlipemias.

Methods: We studied 91 hyperlipemic patients and 19 healthy controls. Hyperlipemic subjects had a diagnosis of polygenic hypercholesterolemia (PH, n=53) or familial combined hyperlipemia (FCH, n=38). Plasma sitosterol, campesterol and lathosterol were determined by gas chromatography coupled to mass spectrometry with multiple selected ion monitoring. To correct for the effect of plasma lipid levels, non-cholesterol sterol concentrations were adjusted for plasma cholesterol (10^2 µmol/mmol cholesterol).

Results: Lathosterol was higher in FCH (125±61´10^2 µmol/mmol) than in PH or controls (95±67 and 88±49, respectively, both p< 0.05). Campesterol was significantly lower in FCH (46±58 ´10^2 µmol/mmol) than in PH or controls (79±81 and 82±59). Sitosterol was significantly higher in PH than in controls (130±81 vs 95±37´10^2 µmol/mmol, p< 0.05). Lathosterol showed a direct relationship with BMI, waist circumference and HOMA (*r .297,* .322, .196, respectively, p< .001) Campesterol and sitosterol had direct relationships with HDL-cholesterol (*r*=0.39 and *r*=0.41, respectively). Multivariate regression analysis showed female gender and sitosterol as independent predictors of HDL-C (*b* -230 and .246, p< .05).

Conclusions: FCH is characterized by increased cholesterol biosynthesis and reduction in absorption; higher cholesterol absorption was found in polygenic hypercholesterolemia. Dietary cholesterol influences HDL-C levels. Investigating cholesterol synthesis and absorption by dosing non-cholesterol sterols is a promising approach to a better insight into the pathogenesis of hyperlipemias.

Poster Session

POTENTIAL ATHEROGENICITY OF POSTPRANDIAL LIPEMIA IN MIXED DYSLIPIDEMIA: THERAPEUTIC TARGET FOR CETP INHIBITION

Z. Julia[1], E. Duchene[1,2], N. Fournier[3,4], N. Bellanger[1], M.J. Chapman[1], W. Le Goff[1], **Maryse Guerin**[1]

[1]UMRS939, INSERM-UPMC, [2]Department of Endocrinology, AP-HP Pitie-Salpetriere, Paris, [3]UMR INRA 1154, University Paris-Sud, Chatenay-Malabry, [4]Service de Biochimie, AP-HP Hopital Europeen Georges Pompidou, Paris, France

Hypothesis: Lipid and cholesterol metabolism in the postprandial phase is associated with both quantitative and qualitative remodelling of HDL particle subspecies that may influence their anti-atherogenic functions in the reverse cholesterol transport pathway.

Methods and results: We evaluated the capacity of whole plasma or isolated HDL particles to mediate cellular FC efflux, CETP-mediated CE transfer and selective hepatic CE uptake during the postprandial phase in subjects displaying mixed dyslipidemia (n=16).

Results: Postprandial large HDL2 displayed an enhanced capacity to mediate FC efflux via both SR-BI- (+12%;$p< 0.02$) and ABCG1- (+31%;$p< 0.008$) dependent pathways in *in vitro* cell systems. In addition, the capacity of whole postprandial plasma (4h and 8h postprandially) to mediate cellular FC efflux via the ABCA1-dependent pathway was significantly increased (+19%; $p< 0.0003$). Concomitantly, postprandial lipemia was associated with elevated endogenous CE transfer rates from HDL2 to apoB-lipoproteins, and with attenuated capacity (-17%;$p< 0.02$) of total HDL to deliver CE to hepatic cells (HepG2) *in vitro*.

Conclusion: Postprandially, cellular cholesterol efflux was preferentially enhanced through both SR-BI and ABCG1 pathways in mixed dyslipidemic subjects. Equally, CETP-mediated transfer of CE to TRL particles was significantly elevated. Moreover hepatic uptake of HDL-CE via SR-BI was diminished. Considered together, these findings strongly suggest that postprandial changes in HDL particle metabolism and function in mixed dyslipidemia may constitute a therapeutic target to attenuate the pro-atherogenic features of postprandial lipemia in dyslipidemic subjects at high cardiovascular risk. Clearly then, CETP inhibition may represent an efficacious strategy in this metabolic context.

Poster Session

EFFECTS OF HIGH DOSE STATIN ON THE HUMAN HEPATIC EXPRESSION OF GENES INVOLVED IN CARBOHYDRATE AND TRIGLYCERIDE METABOLISM

Camilla Pramfalk[1], P. Parini[1], U. Gustafsson[2], S. Sahlin[2], M. Eriksson[3]

[1]Department of Laboratory Medicine, [2]Department of Surgery, [3]Department of Medicine, Karolinska Institute, Stockholm, Sweden

Background: Atorvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis, lowers plasma cholesterol and triglyceride (TG) levels in a dose-dependent manner. Here we aimed to investigate the molecular mechanism(s) that results in lower TG in patients treated with 80 mg/d atorvastatin for four weeks.

Methods: Lipoprotein separation and plasma analysis of lipids, glucose, and insulin were performed. Liver TG mass was determined in pooled samples. Hepatic gene expressions of several genes involved in carbohydrate and TG metabolism were analysed.

Results: Atorvastatin lowered plasma levels of very low density lipoprotein (VLDL) TG (>40%, $p< 0.05$), low density lipoprotein (LDL) TG (N.S.) and liver TG mass compared to placebo. Except for cholesterol changes, no other significant differences in plasma lipids, glucose, and insulin occurred. However, atorvastatin reduced mRNA expressions of sterol regulatory element binding protein 1c (SREBP1c) (>50%, $p< 0.05$), glucokinase (GK) (>75%, $p< 0.05$), angiopoietin-like protein 3 (ANGPTL3) (~30%, $p< 0.01$), and induced mRNA expressions of acetyl-coenzyme A carboxylase 1 (~45%, $p< 0.05$), and glucose-6-phosphatase (G6Pase) (~70%, $p< 0.05$) compared to placebo.

Conclusions: Reduced ANGPTL3 mRNA expression, following treatment with atorvastatin, may contribute to reduced plasma levels of VLDL TG. The reduced liver TG mass together with decreased hepatic SREBP1c and GK expressions and increased G6Pase expression suggests that treatment with atorvastatin do not have negative effects on the insulin-sensitivity in humans. The reduced liver TG mass by high dosage of atorvastatin may be important as a treatment option for fatty liver in humans.

Poster Session

ABC TRANSPORTERS-MEDIATED CHOLESTEROL EFFLUX FROM HUMAN MACROPHAGE FOAM CELLS IS IMPAIRED IN AN ACIDIC EXTRACELLULAR ENVIRONMENT

Miriam Lee-Rueckert[1], J. Lappalainen[1], H. Leinonen[1], M. Jauhiainen[2], P. Kovanen[1]

[1]Wihuri Research Institute, [2]National Public Health Institute and Finnish Institute for Molecular Medicine, Biomedicum, Helsinki, Finland

In the deep microenvironments of advanced human atherosclerotic lesions, the intimal fluid becomes acidic. We hypothesized that an acidic extracellular pH may disturb macrophage-lipoprotein interactions, so compromising cholesterol efflux from these cells. ^{3}H-cholesterol efflux from human monocyte-derived macrophage foam cells to various cholesterol acceptors was evaluated in culture media of pH 5.5, 6.5, and 7.5. It appeared that the lower the pH, the more was cholesterol efflux reduced, the reduction being strongest to lipid-free apoA-I and less so to HDL_2 or to plasma. Of note, cholesterol efflux to apoA-I and HDL_2 at neutral pH was also compromised from foam cells that had been generated at acidic pH. Compatible with this observation, the gene expression of the membrane proteins ABCA1 and ABCG1, which is typically up-regulated by incubation of macrophages with acetyl-LDL in neutral medium, was progressively blocked at acidic pH. ApoE secretion from the foam cells increased at pH 6.5 but was blocked at pH 5.5, so further reducing the cholesterol efflux potential of these cells. In overall, the results revealed that cholesterol efflux is pH-dependent and, moreover, demonstrated multiple mechanisms involved in the inhibitory effect induced by a low pH. The present study discloses a novel effect of the extracellular pH which, by regulating cholesterol efflux mediated by the ABC transporters from macrophage foam cells, may play a critical role in acceleration of atherosclerosis in local areas where the intimal fluid becomes acidic.

Poster Session

IDENTIFICATION OF COMPOUND HETEROZYGOUS DEFICIENCY WITH A NOVEL LARGE DELETION AND Y61X OF THE LIPOPROTEIN LIPASE GENE IN JAPANESE SUBJECT

Atsuko Takagi[1], M. Nagano[2], T. Iwanaga[1], N. Ito[2], H. Hattori[2], T. Egashira[2], Y. Ikeda[1]

[1]National Cardiovascular Center, Suita, [2]BML, Inc., Kawagoe, Japan

Objectives: The early diagnosis of the LPL gene mutations is important for preventing the development of hypertriglyceridemia (HTG) that is one of the defined components of metabolic syndrome. In the Japanese, 24 LPL mutations resulting in non-functional LPL molecule have been reported, in which 20 mutations were accumulated by us. Accumulation of these LPL mutations makes it possible to establish an early diagnostic system of LPL gene mutations. Here we aimed to furthermore identify novel LPL mutations from a subject with HTG.

Methods: A Japanese hypertriglyceridemic subject, #521 (one month, male; TG, 779 mg/dl, TC, 280 mg/dl) was examined. LPL activity and immunoreactive mass in postheparin plasma (PHP) were measured with a selective immunoinactivation assay and MARKIT-M LPL ELISA kit (Dainippon Sumitomo Pharma Co., Japan), respectively. LPL mutations were identified with PCR-SSCP, Invader, Southern blot and DNA sequencing.

Results: Patient #521 showed extremely low levels of LPL mass (1.3 ng/ml) and activity (0.37 unit/h/ml) in PHP. The patient was compound heterozygous deficiency with a novel large deletion (a 54-kb containing 5' upper region and a part of intron 1 in the LPL gene; 53,918 bp from 7594420 to 7648337 in NT-030737.9) and a known nonsense mutation (Y61X) in exon 3 of the LPL gene. We established a simple PCR method to detect this deletion mutation.

Conclusion: Establishment of a simple genetic analysis for the large deletion can promote efficiency for the detection of its LPL gene mutation and may thereby contribute to treatment and prevention of HTG in the Japanese.

Poster Session

LDL RECEPTOR RELATED PROTEIN 1 (LRP1) IS NECESSARY FOR APOLIPOPROTEIN A5 (APOA5) MEDIATED TRIGLYCERIDE REDUCTION

Karoline Brügelmann[1], D. Teupser[2], A. Bartelt[1], P. Gordts[3], J. Heeren[1], M. Merkel[4]

[1]Institute for Molecular Cell Biology, University Hospital Hamburg-Eppendorf, Hamburg, [2]Institute of Laboratory Medicine, University Hospital Leipzig, Leipzig, Germany, [3]Department of Human Genetics, Developmental and Molecular Genetics Section, University of Leuven, Leuven, Belgium, [4]Department of Internal Medicine, Asklepios Clinic St. George, Hamburg, Germany

Objective: ApoA5 is an important regulator of plasma triglyceride levels (TG) in mice and humans. It was proposed that apoA5 activates lipoprotein lipase and thereby accelerates plasma TG hydrolysis. Recent *in vitro* studies suggest that apoA5 may also mediate lipoprotein uptake by members of the LDL receptor family. This study investigates apoA5 interactions with lipoprotein receptors *in vivo.*

Methods: The triglyceride-lowering effect of the human apoA5 transgene (A5tr) was investigated in mice with LDL receptor deficiency ($LDLR^{-/-}$) as well as mice without hepatic LDL receptor related protein 1 (hepatic $LRP1^{-/-}$) and respective littermate wildtype controls (hepatic $LRP1^{+/+}$).

Results: In $LDLR^{-/-}$ mice apoA5 was able to decrease VLDL-TG by 46%. This effect was not augmented by a fat gavage. In addition, apoA5 reduced atherosclerosis on this background on a western type diet by 50% (en face aortic lesions: $LDLR^{-/-}$: 3.2±1.5%; $LDLR^{-/-}$/A5tr: 1.6±0.5%; $p< 0.005$). A similar TG-lowering effect of apoA5 was observed in hepatic $LRP1^{+/+}$ mice ($hLRP1^{+/+}$: 106±29; $hLRP1^{+/+}$/A5tr: 69±26 mg/dl; $p< 0.005$). However, the absence of hepatic LRP1 abolished the effect of the apoA5 transgene in the fasted state (hepatic $LRP1^{-/-}$: 110±20; hepatic $LRP1^{-/-}$/A5tr: 108±22 mg/dl) and under postprandial conditions. All VLDL-TG changes were accompanied by corresponding changes in total TG and VLDL-C.

Conclusion: These data suggest that hepatic LRP1 plays a major role in the apoA5 mediated reduction of plasma TG. Whether this effect is connected to changes in plasma TG hydrolysis, is not clear.

Poster Session

ACTIVE PHOSPHOLIPID TRANSFER PROTEIN (PLTP) REDUCES PROTHROMBIN ACTIVATION AND ATTENUATES BLOOD COAGULATION

C. Oslakovic[1], M. Jauhiainen[2], **Christian Ehnholm**[2], B. Dahlback[1]

[1]Lund University, Malmo, Sweden, [2]National Institute for Health and Welfare, Helsinki, Finland

Background and aim: Rupture of atherosclerotic plaque triggers primary haemostasis events, which involve a cascade of proteolytic reactions resulting in the formation of thrombin and subsequent fibrinogen to fibrin clot conversion. The aim of our study was to elucidate mechanisms that regulate blood coagulation and to determine how PLTP and lipoproteins affect blood coagulation reactions.

Methods: Human apolipoproteins were used together with anionic phospholipids to generate reconstituted lipoproteins, using a detergent solubilization-dialysis method. Blood coagulation was studied by the activation of prothrombin by factor Xa in the presence of factor Va.

Results: The anionic phospholipids lost their procoagulant effect when vesicles were prepared in the presence of apoA-I. The phospholipids of these particles were unable to support binding of factor Va, while binding of prothrombin and factor Xa were efficient. PLTP was shown to efficiently mediate transfer of phospholipids from liposomes to HDL and LDL and to neutralize the procoagulant effect of anionic liposomes. The neutralizing activity was dependent on a catalytically active form of PLTP while the low activity form of PLTP had no effect.

Conclusions: The catalytically active form of PLTP is required to attenuate blood coagulation. ApoA-I was found to neutralize the procoagulant properties of anionic phospholipids by arranging the phospholipids in surface areas that are too small to accommodate the prothrombinase complex. This anionic phospholipid scavenger function may be an important mechanism to control the exposure of anionic phospholipids to circulating blood and thereby prevent inappropriate stimulation of blood coagulation.

Poster Session

SUPPRESSION OF NUCLEAR FACTOR-KAPPAB ACTIVITY IN MACROPHAGES BY CHYLOMICRON REMNANTS: MODULATION BY THE FATTY ACID COMPOSITION OF THE PARTICLES

Clara de Pascale, V. Graham, R.C. Fowkes, C.P. Wheeler-Jones, K.M. Botham

The Royal Veterinary College, Department of Veterinary Basic Sciences,University of London, London, UK

Chylomicron remnants (CMR) induce macrophage foam cell formation, an early event in atherosclerosis. Inflammation also plays a part in atherogenesis and the transcription factor nuclear factor-kB (NF-kB) has been implicated. In this study, the influence of the fatty acid composition of CMR on the activity of NF-kB in macrophages was investigated using CMR-like particles (CRLPs) enriched with SFA, MUFA, n-3/n-6 PUFA and macrophages derived from the human monocyte cell line THP-1. Treatment with n-6 PUFA CRLP resulted in decreased NF-kB luciferase activity and NF-kB activation (measured by DNA binding assay) which was associated with a down-regulation in the expression of phospho-p65-NF-kB and phospho-IkBα (pIkBα). This treatment also reduced secretion of the inflammatory cytokines tumour necrosis factors α, interleukin-6 and monocyte chemoattractant protein-1, which are under NF-kB transcriptional control, and mRNA expression of cyclooxygenase-2, an NF-kB target gene. Further results, comparing CRLPs enriched in SFA, PUFA and MUFA clearly showed that CRLPs enriched in PUFA had a markedly greater inhibitory effect on NF-kB binding to DNA and the expression of phospho-p65-NF-kB and pIkB. Lipid loading of macrophages with CRLPs enriched in PUFA compared with MUFA or SFA also increased the subsequent rate of cholesterol efflux, an effect which may be linked to the inhibition of NF-kB activity. These findings demonstrate that CMR suppress NF-kB activity in macrophages, and that this effect is modulated by the fatty acid composition of the particles. This down-regulation of inflammatory processes in macrophages may represent a protective effect of CMR which is enhanced by dietary PUFA.

Poster Session

NOVEL FORM OF SEVERE COMBINED HYPOLIPIDEMIA EXHIBITS A MUTATION IN THE MASTER REGULATOR OF LIPID METABOLISM SREBP-1

J. Kotzka[1], B. Knebel[1], O. Janssen[2], J. Schaefer[3], S. Mack[4], J. Haas[4], H. Avci[1], **Dirk Müller-Wieland**[4]

[1]German Diabetes Center, Düsseldorf, [2]Endokrinologicum, Hamburg, [3]Philips University Marburg, Marburg, [4]Asklepios Klinik St. Georg, Hamburg, Germany

Objective: Alterations of lipid metabolism play a pivotal role in the development of atherosclerosis and its complications, today's major mortality risks. The predominant regulators controlling cholesterol- and fatty acids synthesis in liver are the sterol regulatory element binding proteins (SREBPs), a family of transcription factors that were formerly identified as cholesterol sensor for LDL-receptor gene expression. Variation of gene structure in these genes might therefore indicate a predisposition to develop complications like myocardial infarction and stroke.

Methods: We investigated 190 unrelated German subjects, including 69 subjects with LDL-cholesterol < 55 mg/dl, for mutations in SREBF-1 and SREBF-2 genes by direct sequencing. The impact on SREBP functionality was analyzed by protein biochemical analyses and promoter reporter gene assays.

Results: A missense mutation in SREBF-1 (P111L) was identified in a subject with LDL-cholesterol < 5 mg/dl. Examination of the subject's family confirmed the mutation in two of three siblings. Detailed clinical evaluation of these subjects disclose a novel form of primary combined hypolipidemia only in SREBP-1a P111L carriers, characterized by low levels of apoB and apoA1 containing lipoproteins, low triglyceride, LDL-cholesterol and HDL-cholesterol levels. Functional analyses indicated that the mutation abolishes phosphorylation of SREBP-1. As a consequence not only transcriptional activation of classical target genes, i.e. LDL-receptor, HMG-CoA reductase, FAS, ABCA1, but also PCSK9 or MTTP, was dramatically reduced.

Conclusions: Phosphorylation of SREBP-1, the master regulator of genes for central rate limiting enzymes of cholesterol and lipid metabolism, appears to be a biological principle with clinical implications.

Poster Session

THE ROLE OF DYSLIPOPROTEINEMIA IN THE TREATMENT OF ISOLATED SYSTOLIC ARTERIAL HYPERTENSION (ISAH) IN GERIATRIC PATIENTS

Tatiana Zubiashvili[1], N. Kipshidze[2]

[1]Geriatric Cardiology, [2]Acad.N.Kipshidze National Center of Therapy, Tbilisi, Georgia

The aim of the work is to estimate the effectiveness of the combined hypotensive and hypolipidemic therapy with calcium antagonist “Norvasc” (“Pfizer”, Germany, daily 5-10 mg) and “Zocor” (“Merck”, Netherlands, daily 20 mg) in comparison to effectiveness of the isolated therapy with “Norvasc” in geriatric patients with ISAH.

The results of the research and the treatment of 107 patients was used as the material for the given work. All patients had undergone the clinical, biochemical, and echocardiographic examination before the treatment and 3 and 6 months after it. Biochemical examination included T-C, LDL- C, VLDL- C, HDL- C, TG.

The combined use of “Norvasc” and “Zocor” gives the stable clinical effect and normalization of the lipid spectrum in 97,3% patients and provides the regression of the left ventricular hypertrophy (LVH) in 54% cases with improvement of the parameters systolic and diastolic function after 3 and 6 month. The isolated hypotensive therapy after 3 months of the treatment gives clinical effect in 67,8% patients and provides the regression of LVH in 36,3% cases without normalization of the lipid spectrum and the stable hypotensive effect after 6 months of the treatment.

The investigation showed, that combined use of “Norvasc” and “Zocor “gives the stable hypotensive effect and regression of LVH with improvement of LV systolic and diastolic function, which is especially important for geriatric patients.

Poster Session

HYPERCHOLESTEROLEMIA (HC) ABROGATES THE BIPHASIC EFFECT OF ANGIOTENSIN II (ANG II) ON ENDOGENOUS CHOLESTEROL SYNTHESIS IN HUMAN MONOCYTES

G. Fóris[1], **Zsolt Sarang**[2], I. Seres[3], G. Paragh[4], Atherosclerosis

[1]First Dept. of Medicine, Medical and Health Center, University of Debrecen, [2]Department of Biochemistry and Molecular Biology, [3]First Dept of Medicine, [4]Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

Objective: Ang II the multifunctional hormone regulates cholesterol biosynthesis in human monocytes. Our aim was to elucidate the concentration dependence of the Ang II effect in monocytes derived from control and HC patients, and their downstream signalling.

Methods: Control and HC monocytes stimulated with Ang II in the presence or absence of different inhibitors.

Results: Our results are as follows: A concentration dependent biphasic effect could only be detected in control monocytes whereas in cells from HC patients only elevated cholesterol synthesis was found. The signal pathway of 10 nM Ang II stimulation involves Ca^{2+} signal, activation of PI3K, MAPK and HMG CoA reductase. In the 500 nM Ang II stimulated control monocytes the suppression of cholesterol synthesis was dependent on the Ca^{2+} signal, the H-7 sensitive cPKC and PI3K, whereas in monocytes from HC pattients only PI3K was involved in increased cholesterol synthesis.

Conclusion: Based on our mRNA determinations in HepG2 cells, we conclude that Ang II signalling in control monocytes leads through PLC - Ca^{2+} translocation - conventional PKC- SOCS3 pathway. In contrast, in monocyes of HC patients is characterized by Ca^{2+} influx, abrogation of H-7 sensitive cPKC, and by PI3K- Ins(345)P3 dependent ξPKC isoform - NFκB - HMG CoA reductase activation.

Poster Session

ONSET OF CORONARY ARTERY DISEASE IN PATIENTS WITH AND WITHOUT FAMILIAL HYPERCHOLESTEROLEMIA IN JAPAN

Atsushi Nohara[1], J. Kobayashi[1], T. Noguchi[1], H. Tada[2], C. Nakanishi[2], M.-A. Kawashiri[2], A. Inazu[3], M. Yamagishi[2], H. Mabuchi[1]

[1]Department of Lipidology, [2]Department of Cardiovascular Medicine, [3]Division of Health Sciences, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

To date, not enough clinical data exist about the age of premature coronary artery disease (CAD) in Japan. Familial hypercholesterolemia (FH) is the typical disease with premature CAD, and we investigated the onset of CAD in Japanese patients with and without FH, before and after strong statin had come to clinical use.

Methods: Consecutive 6730 hospital inpatients in the second department of internal medicine, Kanazawa University Hospital, were analyzed from 1995 to 2005. (First strong statin, atorvastatin, was released in 2000 in Japan.) CAD was defined as confirmed myocardial infarction, or significant coronary stenosis in angiography.

Results: 571 cases (M/F = 421/150, Mean age = 66 ± 11 years) were diagnosed as CAD, among which 120 subjects (M/F=77/43, mean age = 63 ± 13 years) were also diagnosed as heterozygous FH. CAD onset was significantly earlier in FH patients than in non-FH patients (53 ± 10 vs. 65 ± 10 years, $p< 0.0001$), especially in male (49 ± 10 vs. 64 ± 10 years, $p< 0.0001$). This was not significant in female. Onset age became higher after 2000, (60 ± 11 vs. 67 ± 10 years, $p< 0.0001$).

ROC curve analysis showed that CAD onset before 55 years was best to distinguish FH from non-FH in male (sensitivity 0.857, specificity 0.773) with data before 2000.

Conclusion: Our data suggests that CAD before 55 years in male should be recognized as premature CAD in Japan.

Poster Session

METABOLIC BENEFITS OF LIFESTYLE MODIFICATIONS IN PATIENTS WITH METABOLIC SYNDROME

Konstantinos Gkogkos, C. Miggos, O. Siarkou, O. Nikitidou, E. Panara, T. Plougarlis, G. Pavlidis

2nd Internal Medicine Department, General Hospital "G. Papanikolaou", Thessaloníki, Greece

Background and aims: The metabolic syndrome is defined by the presence of at least 3 of the following 5 parameters:

1. fasting plasma glucose ≥110 mg/dl,
2. serum triglycerides ≥150 mg/dl,
3. serum HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women,
4. blood pressure (BP) ≥130/85 mmHg or on BP medication, and
5. waist circumference >102 cm in men and >88 cm in women.

The aim of the present study was to determine the influence of combined diet and exercise on the metabolic profile of patients with metabolic syndrome.

Patients and method: We studied 110 hypertensive patients of an outpatient Hypertension office with BMI 31±3.2 kg/cm^2. The patients were prescribed a low-fat, hypocaloric diet and an exercise program (30 min of aerobic exercise at least 4 times per week). They were clinically and biochemically assessed at baseline, as well as 6 months after initiation of the study. During that period their pharmacological treatment was not modified.

Results: Their weight reduction was 5-8 kg (10.7%) over the six-month period. We recorded mean±sd systolic BP reduction of 10±1.4 mmHg. Regarding the serum lipid profile the following changes were observed: reduction of total cholesterol on the order of 14%, LDL cholesterol by 7.5% and triglycerides by 30%.

Conclusion: The weight reduction and the aerobic exercise had favorable effects on lipid metabolism and the other metabolic parameters and can minimize atherosclerosis and cardiovascular disease risks in patients with metabolic syndrome.

Poster Session

UNFOLDED PROTEIN RESPONSE IN NONALCOHOLIC FATTY LIVER INDUCED BY HIGH FRUCTOSE DIET IN RATS

Hussein Osamah[1], I. Shams[2], M. Shenkman[3], K. Abu Gabal[1], S. Svalb[4], A. Apotovski[5], G. Lederkremer[3]

[1]Department of Internal Medicine, [2]Research Wing, Ziv Medical Center, Safed, [3]Department of Cell Research and Immunology, Tel Aviv University, Tel Aviv, [4]Deparment of Pathology, [5]Department of Psychiatry, Ziv Medical Center, Safed, Israel

Activation of unfolded protein response (UPR) has been implicated in the pathogenesis of insulin resistance and diabetes. In nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) patients, eIF2α phosphorylation is increased in the liver, with failure to activate downstream pathways.

The aim of the present study is investigate the UPR in early stages of experimental NAFL.

Methods: Sprague- Dawley rats were fed with chow diet (*Chow* group); high-cholesterol diet (*Chow+Chol* group); Fructose- enriched diet (*FED* group); FED with high-cholesterol diet (*FED+Chol* group). Livers tissue lysates were subjected to immunoblotting for protein analysis.

Results: CHOP is an important PERK-regulated UPR target gene that is considered as a proapoptotic transcription factor. CHOP induces dephosphorylation of eIF2α to maintain protein synthesis during ER stress. A notable increase in CHOP expression was noticed in cholesterol-enriched diet groups. **Phospho-eIF2α** acts as a protein synthesis down-regulator by inhibiting the translation of most mRNAs. An increase in eIF2α phosphorylation was observed in cholesterol-enriched diet groups. **ATF-6** is dissociated from BiP and it translocated to Golgi apparatus upon ER stress, where it is cleaved, releasing the N-terminal cytosolic part of the protein. An approximately two fold increase in N-terminal ATF-6 was observed when cholesterol was added either to FED or to chow diet.

Conclusion: UPR system is activated in the early stages of NAFL development. eIF2α phosphorylation increased with downstream activation as measured by CHOP upon cholesterol enrichment in the chow and FED groups. ATF-6 activation was also demonstrated but downstream effect is yet to be determined.

Poster Session

SERUM PARAOXONASE ACTIVITY AND ADIPOKINES IN RENAL TRANSPLANTED PATIENTS

Ildikó Seres[1], L. Lőcsey[2], L. Szabó[2], F. Sztanek[1], L. Asztalos[2], J. Padra[1], G. Paragh[1]

[1]First Department of Medicine, Medical and Health Center, University of Debrecen, [2]Department of Surgery, University of Debrecen, Debrecen, Hungary

Increased oxidative stress and inflammation are associated with atherosclerotic coronary disease in renal transplanted patients. HDL-associated paraoxonase (PON1) can prevent LDL oxidation by hydrolysing lipid peroxides in the lipoproteins, therefore it may protect against the development of atherosclerosis. Our aim was to investigate the relationship between serum PON1 activity, renal function, adiponectin, leptin and asymmetric dimethylarginine (ADMA) levels in renal transplanted patients.

Methods: 78 renal transplanted patients and 53 healthy controls were included in the study. We examined fasting serum creatinine, cystatin C, homocysteine, CRP, glucose and lipids. PON1 activity was determined spectrophotometrically. Serum adiponectin, leptin and ADMA levels were measured by ELISA.

Results: We devided our patients (n=78) into two groups according to BMI. Obese patients (BMI>30 kg/m2, n=16) had significantly higher CRP and glucose levels compared to malnourished patients (BMI< 25 kg/m2, n=30). Serum homocystein concentration was elevated compared to control, but similar in the patient groups. Serum ADMA and adiponectin levels were significantly higher in patient groups compared to control ($p< 0.001$). There was significant difference in adiponectin concentration between malnourished and obese transplanted patients ($p< 0.05$). Patients had significantly higher leptin level compared to controls with similar BMI. PON1 activity was higher in patients than in controls ($p< 0.001$). We found significant negative correlation between PON1 activity and CRP level ($p< 0.01$). There were no correlations between PON1 and adiponectin or ADMA levels.

Conclusion: Decreased PON1 activity and nutritional status with altered adipokine levels may be additional factors contributing to premature atherosclerosis in renal transplanted patients.

Poster Session

INCREASED PLASMA RESISTIN CONCENTRATIONS ARE ASSOCIATED WITH ATHEROGENIC SMALL, DENSE LOW-DENSITY LIPOPROTEINS IN PATIENTS WITH TYPE-2 DIABETES

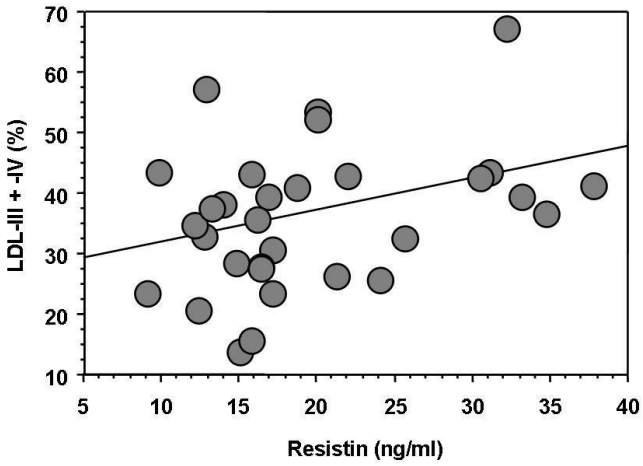
Manfredi Rizzo[1], B. Amann-Vesti[2], C. Zwimpfer[3], G. Spinas[3], K. Berneis[3]

[1]Division of Internal Medicine, University of Palermo, Palermo, Italy, [2]Division of Angiology, [3]Division of Endocrinology, Diabetes and Clinical Nutrition, University Hospital Zurich, Zurich, Switzerland

Background: Resistin was originally proposed in animal models as a link between obesity and insulin resistance, but later studies in humans have shown a divergent role. Yet, resistin seems to be involved in the development of atherosclerosis in humans by promoting the formation of foam cells; further, its expression is induced by oxidized low-density lipoproteins (LDL) in human macrophages.

Methods: We assessed the relationships between resistin and markers of insulin resistance and atherogenic dyslipidemia, including small, dense LDL, in subjects with type-2 diabetes (n= 31, age: 67±10 years, BMI: 28±3 kg/m^2). Plasma resistin was assessed by ELISA and LDL size and subclasses by non-denaturing gradient gel electrophoresis of whole plasma. Correlation analysis was performed using the Spearman rank correlation method.

Results: No associations were found between resistin and age, BMI, waist and hip circumferences as well as markers of insulin resistance, including fasting or postprandial glucose, insulin, HOMA and HbA1c, with the exception of a significant association with C-peptid levels (r= +0.435, p< 0.05). Further, no associations were found between resistin and plasma lipids or LDL size. Regarding LDL subclasses, resistin was inversely associated with larger LDL-I (r= 0.414, p< 0.05) and positively with smaller, denser LDL-III and -IV (r= +0.345, p=0.05, see Figure).



[Figure 1]

Conclusion: These findings suggest that increased plasma resistin concentrations may be associated with atherogenic small, dense LDL in subjects with type-2 diabetes. Yet, whether these findings affect the atherogenic process and clinical end-points in this category of patients remains to be determined by future prospective studies.

Poster Session

MUTANT APO A-I (L178P) IDENTIFIED IN HDL FROM HETEROZYGOTES OF A FAMILY WITH ENDOTHELIAL DYSFUNCTION AND INCREASED ARTERIAL WALL THICKNESS

Helen Karlsson[1], S. Sundberg[1], J.H.M. Levels[2], M. Turkina[3], G. Daniel[4], A. Chroni[4], J.A. Kuivenhoven[2], G.K. Hovingh[2], A.G. Holleboom[2], M. Lindahl[1]

[1]Occupational and Environmental Medicine, Linkoping University, Linkoping, Sweden, [2]Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands, [3]Department of Cell Biology, Linkoping University, Linkoping, Sweden, [4]Institute of Biology, National Center for Scientific Research, Demokritos, Athens, Greece

Heterozygosity for the novel L178P apo A-I mutation underlies reduced plasma HDL cholesterol/apo A-I levels and is associated with increased risk of CAD. It is unclear whether the mutated apo A-I is secreted into plasma and if the mutation is associated with altered protein profiles in HDL.

HDL and LDL/VLDL of 3 L178P heterozygotes and 3 matched controls was isolated by ultracentrifugation and proteins were separated by two-dimensional gel electrophoresis. Apo A-I was analyzed after enzymatic (Glu C) cleavage by MALDI-TOF mass spectrometry (MS). Peptide sequencing was performed by electrospray triple quadrupole MS/MS and nanoLC MS/MS. The HDL antioxidant/anti-inflammatory properties were assayed by an *in vitro* fluorometric method.

Apo A-I peptide containing the L178P mutation, identified by peptide sequencing, was only found in HDL from the heterozygotes The intensity of the mutant peptide was about 30 % of the wild type peptide. Protein profiling showed less apo A-I, apo E and more alpha-1-glycoprotein in HDL and less apo E, apo A-I and apo C-III in LDL/VLDL in the heterozygotes than the controls. In addition, heterozygotes had oxidized/proinflammatory HDL in contrast to control subjects.

Our results show that apo A-I L178P heterozygotes express the mutant apo A-I along with wild type apo A-I, which may impact the atheroprotective functions attributed to HDL. In addition to lower HDL cholesterol, this was associated with lower apo A-I and apo E levels in both HDL and LDL/VLDL and oxidized/proinflammatory HDL. Taken together, these factors may contribute to the atherogenic profile seen in this family.

Poster Session

MORPHOLOGICAL AND FUNCTIONAL CHANGES IN LIVER MITOCHONDRIA OF RATS TREATED WITH HIGH ATORVASTATIN DOSES AND HYPERCHOLESTEROLEMIC DIET

Juan Díaz-Zagoya[1], I. Juárez-Rojop[2], A.M. Zetina[2], J. Ble-Castillo[2], T. Ramón-Frias[3], A. Rodriguez-Hernández[2]

[1]Biochemistry, National University of Mexico, Mexico City, [2]Ciencias de la Salud, Universidad Juarez Autonoma de Tabasco, Villahermosa, [3]Unidad Multidisciplinaria de Comalcalco, Universidad Juarez Autonoma de Tabasco, Comalcalco, Mexico

Atorvastatin (AT) has as main indication decreasing serum cholesterol levels by blocking mevalonate synthesis. It can also decrease other intermediary products of this pathway as coenzyme Q10, a lipidic electron transporter of the mitochondrial respiratory chain. We analyzed the effect of high daily AT doses equivalent to 1.33 (AT1), 2.66 (AT2) and 5.32 (AT3) mg/Kg body weight, given orally during 30 days, on liver mitochondria from Wistar rats with a 2% cholesterol, 0.06% deoxycholate diet. Animals with normal (CD) or hypercholesterolemic (HD) diets were used as controls. After sacrifice, microscopic observation of liver slices dyed with three different techniques was done. Liver mitochondria respiratory conditions and morphology were evaluated by an oxygen monitor and by electron microscopy, respectively. AT treatment improved fatty liver and also produced a moderate hyperglycemic effect, as well as body weight increase. The respiratory control was 2.9±0.10, 1.8±0.11, 2.6±0.05, 2.5±0.05 and 2.3±0.05 for CD, HD, AT1, AT2 and AT3, respectively. The hypercholesterolemic diet produced a fatty liver with a fat gradient distribution from portal zone to central vein, in addition, induced mitochondrial disruption with vesicle formation probably originated from internal mitochondrial membrane. In conclusion, the mitochondrial damage diminished progressively in AT1, AT2 and AT3 livers. Nevertheless, respiratory control decreased with high AT doses.

Poster Session

VERY HIGH INTAKE OF PLANT STANOL ESTERS. TIME FOR REVISION OF EFFICACY AND SAFETY?

Piia Simonen[1], M. Hallikainen[2], M. Nissinen[1], T. Miettinen[1], H. Gylling[2]

[1]Department of Medicine, University of Helsinki, Helsinki, [2]Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland

It is suggested in general that there is little additional cholesterol-lowering effect of plant stanols at daily doses higher than 2-3 g. However, the efficacy and safety of plant stanol intake beyond 4 g/d is unexplored.

We investigated the efficacy and safety of 8.8 g/d of plant stanols as esters (staest) for 10 weeks in a randomized, double-blind, parallel study in mildly hypercholesterolemic subjects (staest, n=25, controls, n=24). Serum and lipoprotein lipids, safety variables, carotenoids, fat-soluble vitamins, and serum non-cholesterol sterols were assayed.

Staest was well tolerated, and safety variables were unchanged. Total and LDL cholesterol levels were reduced by 13 and 17% from baseline, and by 12 and 17% from controls ($P < 0.01$ for all). Synthesis marker lathosterol was increased by 28 %, while serum plant sterols decreased up to 63 % compared with controls ($P < 0.001$ for both). Serum plant stanols increased compared with controls (e.g. serum sitostanol during intervention, controls:16±1 µg/dL, staest: 37± 2 µg/dL, $P < 0.001$), but were normalized in 4 weeks. Staest reduced serum beta-carotene concentration and ratio to cholesterol, but the end product, vitamin A level, was unchanged.

In conclusion, very large plant stanol intake was more effective than the presently recommended 2-3 g of plant stanols/d. Serum plant stanol levels remained at comparable low levels as in studies with lower daily intake, and their quick normalization suggested that large intake of plant stanols might not increase their systemic availability. Accordingly, plant stanol intake can safely be increased with enhanced cholesterol lowering.

Poster Session

IS CHOLESTEROL TEST STRIP A GOOD TOOL FOR CVD RISK SCREENING ?

Sylvie Mabille[1], J.F. Cambier[2], A. De Meester[2], O. Marcovitch[2], O. Descamps[1]

[1]Centre de Recherche Médicale de Jolimont, [2]Département de Cardiologie, Hopital de Jolimont, Haine Saint-Paul, Belgium

Introduction: CardioCheck is an alternative technique to measure lipid values from blood capillary sample by test strip with the advantages of easy feasibility and quick results. We evaluated how such lipid measurements correlate with those obtained by laboratory routine tests and how the non fasted circumstances whereby patients visits the outpatient clinics affects these lipid levels.

Methods: Fifty-six patients hospitalized in various clinical units (mean age: 62 years; 42 men and 14 women) were examined for 2 dosages of lipid levels (TC, LDL, HDL and triglycerides) at 8H00 (fasted) and at 14H00 (after lunch) by the two methods. These lipid levels were then compared for their correlation by linear regression analysis.

Results: The correlations between lipid levels analysed by CardioCheck and by laboratory routine test were rather weak (R^2 varied from 0,46 to 0,69) with very low concordance specially for HDL-C, LDL-C and triglycerides. The blood lipid levels measured by laboratory routine tests, were relatively constant during the day (R^2 of correlation varied from 0,90 to 0,93), except triglycerides (R^2 = 0,65).

Conclusion: We conclude that lipid values obtained by CardioCheck are not really reliable for cardiovascular risk evaluation or follow-up on lipid lowering therapy. In contrast, analyses of lipid levels by laboratory routine tests could be performed at any time of the day rather than to delay the analysis in order to have the patient fasted.

Poster Session

ROSUVASTATIN PROMOTES ANTIOXIDANT EFFECT THROUGH NITRIC OXIDE PATHWAY AND REDUCES SERUM LEVELS OF OXIDIZED-LDL/B2GPI COMPLEXES IN PATIENTS WITH DIABETES MELLITUS

Luis Lopez[1], P. Ames[2], J. Batuca[3], I. Garcia De La Torre[4], K. Kojima[5], E. Matsuura[6]

[1]Corgenix, Inc, Broomfield, CO, USA, [2]Rheumatology and Haematology, Airedale General Hospital, Steeton, UK, [3]Pharmacology, New University of Lisbon, Lisbon, Portugal, [4]Immunology and Rheumatology, Hospital General de Occidente, Zapopan, Mexico, [5]Medical and Biological Laboratory Co, Ltd., Ina, [6]Cell Chemistry, Okayama University Graduate School of Medicine, Okayama, Japan

Introduction: In vivo lipid peroxidation promotes the oxidative modification of low-density lipoprotein (oxLDL) and its binding to β2-glycoprotein I (β2GPI). OxLDL/β2GPI complexes have been implicated in atherogenesis and associated with coronary artery disease and adverse cardiovascular outcomes.

Objective: Because diabetic (DM) patients present elevated titers of these complexes than age/sex matched controls, we evaluated whether Rosuvastatin suppressed the formation of oxLDL/β2GPI complexes and if antioxidant mechanism(s) were involved.

Results: One-hundred eleven DM patients (80F/31M), mean age 54.7±11.0 years, and mean disease duration of 7.7±6.7years were studied. Seventy-six patients received 10 mg of Rosuvastatin daily for 6 weeks and 35 patients that received no Rosuvastatin were used as controls. The mean serum level of oxLDL/β2GPI decreased 33.7% in DM patients after Rosuvastatin treatment (0.80±0.49 to 0.53±0.36units/mL, p< 0.001) compared to a 9.5% variation (0.84±0.62 to 0.76±0.79units/mL, p=0.573) in controls. Rosuvastatin also lowered mean cholesterol (24.7%, p< 0.001), LDL (36.6%) and triglyceride (27.9%) levels. Rosuvastatin-induced reduction of oxLDL/β2GPI complexes was independent of the lipid-lowering effect. Male DM patients had significantly higher baseline oxLDL/β2GPI (0.95±0.52units/mL) than female patients (0.71±0.35units/mL, p=0.008). The correlation of baseline oxLDL/β2GPI serum levels with triglycerides was r=0.325, p=< 0.001, total cholesterol r=0.207, p=0.03, and CRP r=0.196, p=0.039. Serum activity of HDL-associated PON remained unchanged while ADMA, NO2 and NO3 significantly decreased after Rosuvastatin treatment.

Conclusion: Rosuvastatin significantly decreased serum levels of oxLDL/β2GPI complexes suggesting an antioxidant activity. Measuring oxLDL/β2GPI complexes may be a useful modifiable marker in the serologic assessment of atherosclerotic vascular disease in DM.

Poster Session

INFLUENCE OF FENOFIBRATE, SIMVASTATIN AND/OR EZETIMIBE ON CORRELATION OF LDL AND NON-HDL CHOLESTEROL WITH APOLIPOPROTEIN B IN MIXED DYSLIPIDEMIC PATIENTS

M Farnier[1], W. Taggart[2], Q. Dong[2], A. Shah[2], P. Brudi[3]

[1]Point Medical Rond Point de la Nationmedical, Dijon, France, [2]Merck & Co., Inc., Rahway, [3]Merck & Co., Inc., Whitehouse Station, NJ, USA

Correlations between LDL-cholesterol(LDL-C), non-HDL cholesterol(n-HDL-C) and apolipoprotein B(apoB) change after statin therapy in hypercholesterolemic patients. This post-hoc analysis studied the correlations between these parameters in patients with mixed dyslipidemia before and after receiving lipid lowering treatment. Data from 2 randomized double-blind studies with 1112 patients with mixed dyslipidemia receiving treatment [ezetimibe 10mg(E), ezetimibe/simvastatin 10/20mg(E/S), fenofibrate 160mg(F), or ezetimibe/simvastatin/fenofibrate 10/20/160mg(E/S/F)] were pooled. Simple linear regression (SLR) analyses were performed at baseline and after 12 weeks of therapy in the pooled population and by baseline triglyceride (TG) levels (< 2.8 and >=2.8mmol/L). Both LDL-C and n-HDL-C are closely correlated with levels of apoB at baseline, and correlations improved after treatment. Using the fitted SLR model, the average LDL-C and n-HDL-C levels corresponding to an apoB of 0.9g/L were calculated (Table). For TG >=2.8mmol/L, the corresponding LDL-C was generally lower than for TG < 2.8mmol/L, except for fenofibrate. These mean data suggest that to achieve a level of apoB < 0.9g/L in patients with mixed dyslipidemia, treatment goals should be more aggressive with LDL-C targets around 2.1-2.3 mmol/L and for non-HDL-C about 2.8mmol/L in these high risk patients.

	Baseline	Results after 12 weeks of treatment (mmol/L)				
apoB=0.9g/L	n=1092	F n=362	E n=175	E/F n=175	E/S n=179	E/S/F n=179
LDL-C =	2.64	2.72	2.31	2.46	2.07	2.25
non-HDLC =	3.54*	3.03	2.98	2.90	2.69	2.72
n: number of patients with paired obervations;*n=1093						

[Table]

Poster Session

EFFECT OF PUFA N-3 ON LDL SUBFRACTIONS AND SOME NOVEL RISK FACTORS FOR ISCHEMIC HEART DISEASE

Magdalena Dušejovska, M. Vecka, M. Zeman, E. Tvrzicka, J. Kodydkova, L. Vavrova, A. Zak

4th Department of Internal Medicine, Charles University in Prague, 1st Faculty of Medicine, Prague, Czech Republic

Background: Polyunsaturated fatty acids of n-3 family (PUFA n-3) are used in primary and secondary prevention of ischemic heart disease (IHD) due to their pleiotropic beneficial effects. Aim of study was to investigate the effect of PUFA n-3 on some novel risk factors for IHD (LDL subfractions, Lp(a), oxidative stress, homocysteine).

Patients and methods: We enrolled 36 persons in statin group (24M/12F) and 24 untreated probands (15M/9F) into the study. Some patients had isolated/mixed hypertriacylglycerolemia (TAG > 1.7 mmol/l). After 6 week of placebo period (mainly oleic acid), the patients were supplemented with 3g/day of PUFA n-3. Plasma lipoproteins were separated by ultracentrifugation; LDL subfractions were analyzed by Lipoprint LDL System (Quantimetrix).

Results: After PUFA n-3 period, all patients had lower concentrations of plasma and VLDL triacylglycerols, total homocystein, conjugated dienes in LDL and increased concentration of HDL-C. In subgroup of hyper Lp(a) (> 0.25 g/l) individuals, we observed decrease in Lp(a). Concentrations of small dense LDL (sdLDL) were not changed; however, in those with higher content of sdLDL, we observed decreased content of cholesterol in sdLDL. These changes were accompanied with higher activity of paraoxonase-1 and lower activity of glutathion-reductase.

Conclusion: Beneficial effect of PUFA n-3 on metabolic risk factors for IHD include modulation of oxidative stress, the decrease in plasma triacylglycerols, Lp(a) and total homocysteine as well as rise in HDL-C. The effect on sdLDL is more pronounced in persons with pathologic values.

Study was supported by the Research Project MSM 0021620820

Poster Session

LOW HDL-APO AI CLEARANCE ASSOCIATED TO INCREASED HDL3A AND HDL3B CHOLESTEROL PLASMA CONCENTRATIONS IN NEW ZEALAND RABBITS WITH PARTIAL NEPHRECTOMY

Oscar Perez-Mendez[1], P. Toledo-Ibelles[1], M. Franco[2]

[1]Molecular Biology, [2]Nephrology, Instituto Nacional de Cardiologia, Mexico City, Mexico

Background and aim: The renal tubule is the main catabolic site for HDL-apoAI. Moreover, it has been suggested that small HDL are rapidly catabolized. This study aims to establish whether the reduction of functional renal mass induces a selective accumulation of small HDL particles.

Methods: New Zealand White rabbits underwent a chirurgical nephretomization of the left kidney and 50% reduction of the functional mass of the right kidney by arterial coarctation. HDL-apo AI was labeled with ^{125}-I and its clearance was monitored in plasma during 72h. HDL subclasses were separated by PAGE in native conditions. The size distribution was determined by enzymatic cholesterol staining of the electrophoresis gels followed by densitometric analysis. The absolute cholesterol concentrations of HDL subclasses were estimated using the relative percentage and the HDL-cholesterol plasma concentration.

Results: Creatinine in nephrectomized rabbits increased about 50% in 4 weeks after the chirurgical intervention without proteinurie. Plasma HDL-cholesterol drastically increased (75%) associated to a 140% increase of the cholesteryl esters plasma concentrations of the HDL3a and HDL3b. HDL-apo AI clearance was lower at the first phase of the dye-away curve. In nephrectomized rabbits, HDL2b, 2a and 3a subclasses were cleared slower than in sham animals up to 12h after the injection of the radiolabeled HDL.

Conclusions: The reduction of renal functional mass induced an HDL-cholesterol increase with a selective accumulation of cholesteryl esters in HDL3a and 3b as a result of an impaired apo AI clearance. These results should be interpreted by considering an intravascular inter-conversion of HDL subclasses.

Poster Session

EZETIMIBE WITH OR WITHOUT SIMVASTATIN INCREASES SMALL DENSE LOW-DENSITY LIPOPROTEINS IN HEALTHY MEN - A RANDOMIZED TRIAL

I. Gouni-Berthold[1], M. Rizzo[2], **Heiner K. Berthold**[3], G. Spinas[4], W. Krone[1], K. Berneis[4]

[1]Department of Internal Medicine II, University of Cologne, Cologne, Germany, [2]Department of Internal Medicine and Emerging Diseases, University of Palermo, Palermo, Italy, [3]Charite University Medicine Berlin, Berlin, Germany, [4]Division of Endocrinology, Diabetes and Clinical Nutrition, University Hospital Zurich, Zurich, Switzerland

Objective: To determine the effects of ezetimibe alone, simvastatin alone, and of the combination of both drugs on small dense LDL, assessed by gradient gel electrophoresis.

Methods: Monocenter randomized parallel 3-group open-label study in 72 healthy men with a baseline LDL cholesterol concentration of 111 ± 30 mg/dL. Interventions: 14-day treatment with ezetimibe (10 mg/day, n = 24), simvastatin (40 mg/day, n = 24) or their combination (n = 24). Blood was drawn before and after the treatment period. Main outcome measures: Generalized estimating equations were used to assess the influence of drug therapy on LDL subfraction distribution, controlling for within-subject patterns (clustering). The analyses were adjusted for age, BMI, and baseline concentrations of LDL cholesterol and triglycerides.

Results: Ezetimibe alone or in combination with simvastatin changed LDL subfractions towards a more pro-atherogenic profile (fully adjusted Wald chi square test). It increased small dense LDL proportions (LDL-IVA +14.2%, *P* = .0216 and +28.5%, *P* = .0002; LDL-IVB +16.7%, *P* = .0392 and +14.3%, *P* = .44, alone and in combination with simvastatin, respectively). Larger, more buoyant LDL-I were significantly decreased by ezetimibe and the combination (-13.9%, *P* < .0001 and -7.3%, *P* = .0743, respectively). Simvastatin monotherapy decreased LDL-IVB (-16.7%, *P* = < .0001) and had only minor effects on LDL-I (-4.6%, *P* = .002). None of the treatments had an effect on LDL particle size.

Conclusions: In healthy men, treatment with ezetimibe, alone or in combination with simvastatin, is associated with the development of a pro-atherogenic LDL subfraction profile.

Poster Session

A RANDOMIZED COMPARISON OF ROSUVASTATIN PLUS EZETIMIBE VERSUS SIMVASTATIN PLUS EZETIMIBE: RESULTS OF THE GRAVITY STUDY

Christie M. Ballantyne[1], R. Schiebinger[2], V. Cain[2], on behalf of the GRAVITY Study Investigators

[1]Centre for Cardiovascular Disease Prevention, Baylor College of Medicine and Methodist DeBakey Heart and Vascular Center, Houston, TX, [2]AstraZeneca, Wilmington, DE, USA

Background: Combination therapy may be required in high-risk patients to achieve target LDL-C levels. GRAVITY (NCT00525824 **G**auging the lipid effects of **R**osuv**A**statin plus ezetimibe **V**ersus s**I**mvastatin plus ezetimibe **T**herap**Y**), evaluated efficacy of rosuvastatin (R)10 or 20mg +ezetimibe (E)10mg vs simvastatin (S)40 or 80mg+E10mg in patients with raised LDL-C (130-220mg/dL), triglyceride < 400mg/dL and CHD (or CHD risk equivalent).

Methods: Patients underwent a 6-week dietary lead-in and lipid-lowering medication washout, before 6 weeks of R10mg, R20mg, S40mg, or S80mg monotherapy. E10mg was subsequently added to each regimen for 6 weeks. Primary endpoint in this open-label study was percentage LDL-C change from baseline to week 12.

Results: R20mg+E10mg significantly reduced LDL-C, triglyceride, non-HDL-C, and Apo-B compared with S40mg+E10mg or S80mg+E10mg. More patients achieved LDL-C goals of < 100mg/dL and < 70mg/dL with R20mg+E10mg than with S40mg+E10mg or S80mg+E10mg, and of < 100mg/dL with R10mg+E10mg than with S40mg+E10mg. Except for one myopathy case (S80mg monotherapy), all treatments were well-tolerated.

	Mean % change from baseline					**% of patients achieving LDL-C goals**		**P values**
	LDL-C	Non-HDL-C	HDL-C	TG	Apo-B	≤100 mg/dL	≤70 mg/dL	
R10mg+E10mg (N=210)	-59.7*	-54.7†	6.4	-28.9*	-46.1*	93.3*	67.1	*p≤0.05 vs S40mg+E10mg; †p≤0.001 vs S40mg+E10mg;
R20mg+E10mg (N=204)	-63.5†§	-58.9†§	7.5*‡	-35.0†§	-49.5†§	95.6†‡	77.0†§	*p≤0.05 vs S40mg+E10mg; †p≤0.001 vs S40mg+E10mg; ‡p≤0.05 vs S80mg+E10mg; §p≤0.001 vs S80mg+E10mg
S40mg+E10mg (N=199)	-55.2	-49.9	3.9	-23.0	-42.0	87.4	55.3	
S80mg+E10mg (N=201)	-57.4	-52.4	4.3	-25.8	-44.2	88.6	67.7	

[Table 1]

Conclusion: R20mg+E10mg produced greater improvements in lipid parameters and allowed more patients to achieve LDL-C goals, than S40mg+E10mg or S80mg+E10mg. R10mg+E10mg was more effective and more patients achieved LDL-C < 100mg/dL than S40mg+E10mg.

Poster Session

SUBGROUP ANALYSES FROM UK INPRACTICE STUDY: ACHIEVEMENT OF LDL-C LEVELS WITH THREE DIFFERENT DRUG STRATEGIES AFTER FAILURE OF SIMVASTATIN 40MG

P. Harvey[1], T. Mccormack[2], R. Gaunt[3], **Azhar Ahmad**[4], S. Griffiths[5], P. Robinson[5]

[1]The Crouch Oak Family Practice, Addlestone, [2]Whitby Group Practice, Whitby, [3]Rowden Medical Practice, Chippenham, [4]Schering-Plough, Welwyn Garden City, [5]MSD, Hoddesdon, UK

Background: Primary efficacy results from the INPRACTICE* study involving 786 high-risk CVD patients in 34 UK primary care centres showed that in patients treated with simvastatin (S) 40mg who were not at an LDL-C target of < 2mmol/l , adding ezetimibe (E) to S 40mg significantly improved attainment of this target, compared with switching to atorvastatin (A) 40mg or to rosuvastatin (R) 5-10mg (69% v 34% v 14% respectively, p< 0.001 for E+S v A and v R). We performed pre specified subgroup analysis of the primary efficacy endpoint based on age, diabetes status, baseline LDL-C values and rosuvastatin starting dose (administered in accordance with the UK SPC), and a post-hoc subgroup analysis based on CVD prevention status.

Results: Results for subgroups analysis based on age, diabetes status, baseline LDL-C values, rosuvastatin dose requirement, and CVD prevention status were consistent with the overall study results; more patients achieved LDL-C< 2mmol/l with E+S than with A or R alone. The differences were more marked in the subgroup of patients with baseline LDL-C ≥2.5mmol/l, with the % achieving LDL-C < 2mmol/l being 54%, 18% and 5% respectively (p< 0.001 for E+S v A and v R).

Conclusion: Adding E to S 40mg significantly improved several lipid parameters and attainment of LDL-C < 2mmol/l, compared with switching to A 40mg or to R 5-10mg in the pre specified and post-hoc subgroup analyses of the INPRACTICE study.

*Presented at ESC 2009

Poster Session

INVERSE RELATIONSHIP BETWEEN HDL-C RAISING AND HSCRP REDUCTION IN OLDER PATIENTS TREATED WITH LIPID-LOWERING THERAPY IN THE VYTELD STUDY

W. Brown[1], **J Foody**[2], F. Zieve[3], C. Jones-Burton[4], Q. Dong[4], J. Tomassini[5], A. Shah[4], A. Tershakovec[5]

[1]Emory University School of Medicine, Atlanta, GA, [2]Brigham and Women's Hospital, Boston, MA, [3]McGuire VA Medical Center, Richmond, VA, [4]Merck & Co., Inc, Rahway, NJ, [5]Merck & Co., Inc, North Wales, PA, USA

Background: This exploratory analysis assessed associations between hsCRP and lipid factors in older (>65yrs) patients with moderately-high/high CVD risk, treated with ezetimibe/simvastatin(E/S) or atorvastatin(A).

Methods: Post-hoc analysis of randomized, double-blind 12-wk study. Correlations assessed in 1054 patients with baseline and 12-wk hsCRP ≤10mg/L, pooled across doses of E/S(10/20,10/40mg) and A(10,20,40mg), and pooled E/S+A treatments.

Results: Correlations between baseline levels of hsCRP with LDL-C, non-HDL-C and ApoB were weak and non-significant in the E/S, A and pooled E/S+A groups. After 12-wks of treatment, correlations were significantly higher in all groups. In contrast, HDL-C was negatively and significantly correlated with hsCRP in the A and pooled E/S+A groups at baseline and all groups at 12-wks. Associations between changes in hsCRP and these lipid factors were low but significant for pooled E/S+A, and also for LDL-C and non-HDL-C in the A group and HDL-C in the E/S and A groups.

Conclusions: Relationships between hsCRP and lipid factors were weak at baseline and strengthened somewhat post-treatment in older patients. HDL-C was negatively and consistently correlated with baseline and 12-wk hsCRP levels, and with therapy-induced HDL-C and hsCRP changes.

Spearman Correlation Coefficients	Baseline levels			12 week levels			Changes at 12 weeks		
Relationship hsCRP and	Pooled E/S	Pooled A	Pooled E/S+A	Pooled E/S	Pooled A	Pooled E/S+A	Pooled E/S	Pooled A	Pooled E/S+A
LDL-C	0.018	-0.067	-0.034	0.154‡	0.102†	0.110§	0.061	0.082†	0.078†
HDL-C	-0.091	-0.110‡	-0.103§	-0.128‡	-0.133§	-0.132§	-0.166§	-0.092†	-0.117§
non-HDL-C	0.051	-0.004	0.018	0.185§	0.138§	0.138§	0.094	0.078†	0.085‡
ApoB	0.072	-0.001	0.029	0.177§	0.122‡	0.133§	0.092	0.077	0.083‡

†p<0.05, ‡p<0.01, §p<0.001 for baseline levels, wk 12 levels and/or change at 12 wks; Pooled E/S=ezetimibe/simvastatin 10/20 and 10/40 mg (n=419 for paired endpoints); Pooled A=atorvastatin 10, 20, 40 mg (n ≥627 for paired endpoints); pooled E/S+A (n ≥1046 for paired endpoints)

[Spearman Correlation Coefficients]

Poster Session

HDL CHOLESTEROL RESPONSE TO GROWTH HORMONE REPLACEMENT IS ASSOCIATED WITH COMMON CETP GENE VARIATION (-629C>A) AND MODIFIED BY GLUCOCORTICOID TREATMENT

Robin Dullaart[1], G. van den Berg[1], A. van der Knaap[1], J. Dijck-Brouwer[2], G. Dallinga-Thie[3], P. Zelissen[4], W. Sluiter[1], A. van Beek[1]

[1]Endocrinology, [2]Laboratory Center, University of Groningen, Groningen, [3]Laboratory of Experimental Vascular Medicine, University of Amsterdam, Amsterdam, [4]Endocrinology, University of Utrecht, Utrecht, The Netherlands

Objective: Growth hormone (GH) replacement lowers total cholesterol and low density lipoprotein cholesterol (LDL-C) in GH deficient adults, but effects on HDL cholesterol (HDL-C) are variable. GH and glucocorticoids both decrease cholesteryl ester transfer protein (CETP) activity, which is important in HDL metabolism. We determined the extent to which the changes in HDL-C in response to GH replacement are predicted by the -629C>A CETP promoter polymorphism, and questioned whether this association is modified by concomitant glucocorticoid treatment.

Design and methods: 91 GH deficient adults (63 receiving glucocorticoids) were genotyped for the -629 CETP C>A polymorphism. Fasting serum lipids were measured before and after 1.2 ± 0.4 years of GH treatment (Genotropin, Pfizer Inc.).

Results: In the whole group, total cholesterol and LDL-C decreased ($P< 0.05$) after GH treatment, but the changes in HDL-C were not significant. In CC carriers receiving glucocorticoids (n=19), HDL-C rose by 0.15 ± 0.25 mmol/l ($P=0.02$; $P< 0.03$ from unchanged HDL-C in -629 AA+CA carriers on glucocorticoids and from CC homozygotes not receiving glucocorticoids). Multivariate regression analysis showed that individual changes in HDL-C were predicted by the CETP polymorphism (CC vs. AA+CC, $P=0.006$) in glucocorticoid users, independently of baseline HDL-C and other variables including ApolipoproteinE4 carrier status; an opposite association with the CETP polymorphism was found in patients not receiving glucocorticoids ($P=0.053$).

Conclusions: We suggest a common CETP variant -glucocorticoid treatment interaction concerning the effect of GH replacement on HDL-C. This may explain some of the reported variation in the HDL-C response to GH.

Poster Session

HIGH DENSITY LIPOPROTEIN AND CHOLESTEROL EFFLUX IN PATIENTS WITH TYPE 2 DIABETES

Hong Chang Tan[1], E.S. Tai[2], D. Sviridov[3], P. Nestel[3], C.L.W. Ng[4], Y.H.L. Teo[5], M. Lu[5], E. Chan[6], D.C.H. Wai[1]

[1]Department of Endocrinology, Singapore General Hospital, [2]Department of Medicine, National University of Singapore, Singapore, Singapore, [3]Baker IDI Heart & Diabetes Institute, Melbourne, VIC, Australia, [4]Physiotherapy, Singapore General Hospital, [5]Office of Research, Singapore Helath Services, [6]National University of Singapore, Singapore, Singapore

Objectives: To investigate the roles of high density lipoproteins(HDL) particle size and number on the ability of HDL in patients with type 2 diabetes to support cholesterol efflux from macrophages.

Methods: 45 patients were recruited from the Diabetes Centre in Singapore General Hospital. Lipoprotein molecular profile was studied by nuclear magnetic spectroscopy(NMR). Cholesterol efflux was expressed as percentage efflux of H3-cholesterol in lipid laden THP-1 macrophages incubated with patients' serum depleted of lipoprotein B to provide an HDL-enriched acceptor medium. Correlation analyses were then performed.

Results: Small HDL particles comprised 71% of the total HDL particles. HDL concentration(HDL-C) showed an inverse correlation with Body mass index(BMI)(r=-0.309,p=0.039), waist hip ratio(r=-0.357,p=0.016) and triglyceride levels(r=-0.3,p=0.45). While BMI also had an inverse correlation with large HDL(r=-0.318,p=0.033) and medium HDL particles(r=-0.008,p=0.957), this trends towards positive with small HDL particles(0.145,p=0.341). Increasing HDL-C and ApoA-I was associated with an increasing number of large, but not medium or small HDL particles. Percentage cholesterol efflux showed a positive correlation with HDL-C(r=0.27,p=0.07) and total HDL particle concentration(r=0.283,p=0.63) that did not reach statistical significance. In comparison, although efflux also correlated positively with large HDL particles(r=0.15,p=0.33) and significantly with medium HDL particles(r=0.327,p=0.03), this trend towards an inverse association with small HDL particles(r=-0.066,p=0.67).

Conclusion: Our data suggest that patients with type 2 diabetes have a preponderance of small HDL particles. In these patients, particle number, ApoA-I concentration and HDL-C do not adequately capture the ability of HDL to promote cholesterol efflux since small particles which are poor effectors of cholesterol efflux predominate.

Poster Session

OPTIMIZED ASPIRIN DOSING REGIMEN FOR REDUCTION OF NIACIN-INDUCED FLUSHING

Cynthia Sundell[1], C. Keyserling[1], H. Klepp[1], R. Goldwater[2], R. Scott[1]

[1]Cerenis Therapeutics, Labege, France, [2]PAREXEL Early Phase Unit, Baltimore, MD, USA

Herein we report two clinical studies that evaluate the ability of simulated slow release aspirin (ASA) when administered for sufficient times before and after niacin (NA) to decrease NA-induced flushing. The first study, in 30 subjects, assessed the ability of 4 days of ASA pre-dosing with 81 mg in the AM and 162 mg in the PM, followed by ASA 20 mg/hr for 12 hrs with 500 mg of immediate-release NA at 6hrs. The ASA regimen reduced the maximal severity of flushing (MSF) by 53% (p=0.002) versus placebo. The second study, in 54 subjects, evaluated two ASA dosing regimens (A) and (B) versus placebo for their ability to inhibit flushing associated with a single 2g dose of extended-release (ER) NA. (A): 3 days of pre-dosing with 240 mg of ASA in the PM, followed by ASA 30 mg/hr for 8hrs with ER-NA at 4hrs; (B): no ASA pre-dosing and 10 mg/hr ASA for 6hrs with ER-NA at 6hrs. (A) reduced the MSF compared to placebo by 37% (p=0.0003) whereas (B) had no significant effect. (A) also reduced the MSF compared to (B) by 29% (p=0.013). (A) decreased the Number of Flushing Episodes by 27% (p=0.015) compared with placebo whereas (B) had no significant effect. (A) decreased the Duration of Flushing (DUF) by 42% (p=0.0004) compared to placebo, whereas (B) had no significant effect. (A) also reduced the DUF by 34% compared to (B) (p=0.005). These data demonstrate that the timing and duration of ASA exposure is critical in inhibiting flushing.

Poster Session

THE EFFECTS OF OMEGA-3-POLYUNSATURATED FATTY ACIDS AND ATORVASTATIN ON FATTY ACID STATUS OF BLOOD IN PATIENTS WITH DYSLIPIDEMIA

Albert Galyavich, L. Salakhova

Faculty Therapy and Cardiology, Kazan State Medical University, Kazan, Russia

Objectives: The aim of the study were to examine effects of polyunsaturated fatty acids (n-3-PUFA) and atorvastatin on blood levels of fatty acids in patients with coronary heart disease (CHD).

Methods: 28 patients, 18 men and 12 women, 59.5±7.6 years of age received 1.0 g n-3 PUFA per day for 4 weeks. 20 pts, 9 men и 11 women, 58.2±6.6 years of age received 10 mg atorvastatin daily for 12 weeks. The lipid profile including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) was assessed by conventional method. Circulating fatty acids in a capillary blood were assessed by gas chromatography before and after 4 weeks of treatment with both of drugs. The levels of TC, LDL-C and TG did not differ between groups statistically.

Results: After treatment with atorvastatin level of arachidonate was increased by 41% (from 5.7 ± 0.8% of total fatty acids to 8.4 ± 1.1, $p < 0.01$). There were no significant changes for the main fatty acids concentrations including n-3 PUFA. Level of linoleic acid was tended to decrease (from 21.94 ± 5.40 to 19.17 ± 3.77, $p < 0.099$). Treatment with n-3-PUFA leads to increase in levels of eicosapentaenoic acid (EPA) from 3.00±1.93% to 4.52±2.45%, p=0.015 and docosahexaenoic acid (DHA) from 0.76±0.29% to 0.93±0.31%, p=0.007 and decline in concentration of saturated fatty acids (from 49.61±3.23% to 48.28±1.81%, p=0.043) particularly palmitic acid (from 34.86±3.78% to 32.96±1.95%, p=0.004).

Conclusions: N-3-PUFA increased the levels of EPA and DHA, atorvastatin increased only arachidonic acid concentration.

Poster Session

CHOLESTERYL ESTER TRANSFER PROTEIN AND MORTALITY IN PATIENTS UNDERGOING CORONARY ANGIOGRAPHY

Philipp Eller[1], A. Ritsch[1], H. Scharnagl[2], I. Tancevski[1], K. Duwensee[1], E. Demetz[1], A. Sandhofer[1], B. Boehm[3], B. Winkelmann[4], J. Patsch[1], W. März[5]

[1]Innsbruck Medical University, Innsbruck, [2]Medical University Graz, Graz, Austria, [3]University Hospital Ulm, Ulm, [4]Cardiology Group, Frankfurt-Sachsenhausen, [5]Synlab Center of Laboratory Diagnostics, Heidelberg, Germany

Background: The role of cholesteryl ester transfer protein (CETP) in the development of atherosclerosis is still open to debate. In the ILLUMINATE trial, inhibition of CETP led to increased high-density lipoprotein levels but increased the risk of cardiovascular mortality and morbidity. Here, we present a prospective observational study of patients referred to coronary angiography where CETP plasma levels were related to mortality and morbidity.

Methods: CETP concentration was determined in 3256 participants of the Ludwigshafen Risk and Cardiovascular Health study who were referred to coronary angiography at baseline between 1997 and 2000. Median follow-up time was 7.75 years. Primary and secondary endpoints were all-cause and cardiovascular mortality, respectively.

Results: CETP levels were higher in women and lower in smokers, in diabetic patients, and in patients with unstable coronary artery disease, respectively. In addition, CETP levels were correlated negatively with high-sensitivity C-reactive protein and IL-6. The age and sex-adjusted hazard ratios for death in the lowest CETP quartile was 1.37 (1.10-1.17, p=0.004) compared to patients in the highest CETP quartile. CETP retained prognostic value after further adjustment for classical cardiovascular risk factors, medication and diabetes mellitus with a hazard ratio of 1.33 (1.07-1.65, p=0.011) in the lowest CETP quartile.

Conclusions: We interpret our data to suggest that low endogenous CETP plasma levels *per se* are associated with increased all-cause and cardiovascular mortality questioning the concept of pharmacological CETP inhibition.

Poster Session

CETP EXPRESSION REVERSES THE RECONSTITUTED HDL-INDUCED INCREASE IN VLDL

Yanan Wang[1], J. F.P. Berbée[1], J.A. Kuivenhoven[2], J. W.A. Smit[1], L. M. Havekes[1,3,4], J. A. Romijn[1], P. C.N. Rensen[1]

[1]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center (LUMC), Leiden, [2]Dept. of Vascular Medicine, AMC, Amsterdam, [3]Cardiology, LUMC, [4]TNO-Biosciences, Leiden, The Netherlands

Introduction: Studies in mice indicated that rHDL infusion adversely affect VLDL levels, but this effect is less apparent in humans. This discrepancy may be explained by the fact that humans, in contrast to mice, express the cholesteryl ester transfer protein (CETP).

Aims: To investigate the role of CETP in the effects of rHDL on VLDL metabolism by using *APOE*3-Leiden (E3L)* and *E3L.CETP* mice.

Methods: Female *E3L* and *E3L.CETP* mice were fed a Western-type diet for 3 weeks. Subsequently, *E3L* and *E3L.CETP* mice received a single intravenous injection of rHDL or vehicle. Blood was drawn before and 1, 8, 24, 48 and 72 hours after injection.

Results: At 1 h after injection, rHDL significantly increased plasma cholesterol (C) in both *E3L* (+63%) and *E3L.CETP* mice (+28%) via increasing VLDL-C and HDL-C in E3L mice and HDL-C only in E3L.CETP mice. rHDL increased plasma triglycerides (TG) in *E3L* mice (+89%), but not in *E3L.CETP* mice, explained by competition of rHDL with VLDL for LPL-mediated TG hydrolysis. At 24 h after injection, plasma cholesterol in *E3L* mice treated with rHDL was still significantly higher than vehicle (+33%), whereas decreased in *E3L.CETP* mice (-27%). In *E3L* mice, rHDL strongly increased TG (+67%), explained by increasing hepatic VLDL-TG production. In contrast, rHDL didnot affect TG in *E3L.CETP* mice, because of rapid remodelling of newly synthesized VLDL by CETP.

Conclusions: CETP protects against the rHDL-induced increase in VLDL. We anticipate that treatment of cardiovascular disease by rHDL shouldnot be combined with concomitant CETP inhibition.

Poster Session

HEPATIC LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1 (LRP1) INCREASES CHYLOMICRON REMNANT UPTAKE BY TWO MECHANISMS

Alexander Laatsch, U. Beisiegel, J. Heeren

University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Objective: The low density lipoprotein receptor-related protein 1 (LRP1) binds to apolipoprotein E (apoE) and internalises postprandial chylomicron remnants (CR). We showed previously that insulin has an impact on the postprandial lipid metabolism, as it leads to a translocation of LRP1 into the plasma membrane. This translocation promotes the uptake of apoE containing CR primarily into the liver. In case of a lipid-rich meal, the plasma apoE pool may not be sufficient to mediate the uptake of all CR. As we could demonstrate earlier, the HDL-bound apoE plasma pool can be refilled through the recycling of apoE from hepatocytes. We now investigated whether this HDL-induced recycling process is facilitated via LRP1.

Methods: Experiments were carried out in HEK293 wild-type cells with and without transfection of human full-length LRP1 or siRNA knockdown of LRP1. The recycling rate of apoE from CR/apoE was determined using fluorescent and radioactive labels. Additionally, confocal live-imaging and microscopy of fixed samples were performed.

Results: After induction with HDL, recycling of apoE from LRP1 containing endosomes was observed. The overall recycling rate was reduced in LRP1-knockdown cells. In these cells with reduced LRP1 levels, especially the HDL-inducible fraction of the recycling process was impaired.

Conclusions: Additionally to the insulin-dependent LRP1 translocation, which enhances the uptake rate for CR, LRP1 is responsible for HDL-induced recycling of apoE. This specific recycling route is different from the basal apoE efflux mechanism. Together with the insulin-dependent, enhanced uptake, LRP1 ensures a high postprandial apoE turnover facilitating the efficient clearance of CR.

Poster Session

DEFECTIVE TG CATABOLISM AND SUBSTRATE OXIDATION IN MICE LACKING CGI-58 IN OXIDATIVE TISSUES

Kathrin Anna Zierler, D. Jaeger, N.M. Pollak, R. Zechner, G. Haemmerle

Institute for Molecular Biosciences, Karl Franzens University, Graz, Austria

Triglycerides (TG) are the major energy fuel in mammals. The mobilization of adipose and non-adipose tissue TG stores is catalyzed by lipases. Defects in this process can be associated with the development of metabolic disorders like obesity, type II diabetes, hepatosteatosis and myopathy. Since adipose triglyceride lipase (ATGL) plays a key role in the mobilization of cellular TG stores, mutations in the ATGL gene provoke multiple TG accumulation in mice and humans. Furthermore, it has been shown that ATGL TG hydrolysis is increased by the presence of comparative gene identification-58 (CGI-58). Human mutations of the CGI-58 gene are known to cause Chanarin Dorfman Syndrome (CDS), a rare disease characterized by systemic TG accumulation, hepatosteatosis, myopathy and ichthyosis.

In this study, we focused on the role of CGI-58 in lipid metabolism in non-adipose tissue. Since CDS patients suffer from hepatosteatosis and myopathy, we generated mice lacking CGI-58 specifically either in the liver or in muscle to elucidate the role of this protein in highly oxidative tissues. The hepatocyte-specific loss of CGI-58 causes an excessive TG accumulation in the liver due to impaired TG hydrolysis as well as reduced fatty acid oxidation. Mice lacking CGI-58 specifically in cardiac and skeletal muscle show drastically increased lipid content in the heart leading to a severe myopathy. Interestingly, plasma TG and free fatty acid levels are only slightly altered in both mouse lines. Taken together, our findings demonstrate the importance of CGI-58 in energy metabolism in oxidative tissues.

Poster Session

REVERSE CHOLESTEROL TRANSPORT IS NOT INFLUENCED BY HDL COMPOSITION OR THE REDUCED MACROPHAGE ABCG-1 EXPRESSION IN CHRONIC KIDNEY DISEASE RATS

J.T. Machado, R.S. Pinto, G. Castilho, A. Machado-Lima, L.S. Okuda, R.T. Iborra, J.C. Rocha, A.B. Carreiro, E.R. Nakandakare, S. Catanozi, **Marisa Passarelli**

Lipids Laboratory (LIM10), Faculty of Medical Sciences of the University of Sao Paulo, Sao Paulo, Brazil

Objectives: Atherosclerosis is prevalent in chronic kidney disease (CKD) and associated with disturbances in the reverse cholesterol transport. In CKD rats we analyzed the HDL composition and its ability to remove cell cholesterol as well as the macrophage ABCG-1 expression.

Methods: Plasma urea, creatinine, total cholesterol (TC), triglycerides (TG) and glucose and urinary protein excretion (UPE) were determined before and 60 days after CKD induction by 5/6 nephrectomy in male Wistar rats (n=18) and in control sham operated animals (C;n =8). Plasma anti-carboxymethyllysine (anti-CML) was determined by ELISA. To determine the cell cholesterol efflux rate ^{14}C-cholesterol and LDL-enriched macrophage were incubated along time with HDL (50 µg/mL) from C or CKD rats. Macrophage ABCG-1 expression was determined by immunoblot. Comparisons between groups were done by Student's t test.

Results: TC, TG, urea and UPE increased after 60 days in CKD rats. The anti-CML levels were similar CKD and C rats. HDL contents of total protein, phospholipids, TC and TG enhanced in CKD compared to C. In spite of an 11% reduction in the ABCG-1 expression in the CKD-macrophages (p= 0,0003) no differences were found in the HDL-mediated ^{14}C-cholesterol macrophage efflux rate between C and CKD.

Conclusion: In CKD rats in spite of a reduced macrophage ABCG-1 expression and of an altered HDL composition the ability of the latter to remove cell cholesterol is not modified.

Funding: FAPESP, Brazil

Poster Session

DIETARY MILK PHOSPHOLIPID IMPROVES DIET-INDUCED HEPATIC STEATOSIS IN MICE BY REDUCING INTESTINAL CHOLESTEROL ABSORPTION

A. Kamili[1], E. Wat[1], S. Tandy[1], W.S.R. Chung[1], A. Brown[2], M. Rowney[2], **Jeffrey S Cohn**[1]

[1]Nutrition and Metabolism Group, Heart Research Institute, Sydney, NSW, [2]Murray Goulburn Nutritionals Ltd, Melbourne, VIC, Australia

Introduction: Work in our laboratory has shown that dietary milk phospholipid (PL) can reduce hyperlipidemia, hepatomegaly and hepatosteatosis in high-fat-fed mice. Reduction in liver triglyceride was associated with a decrease in the expression of liver fatty acid synthesis genes. The mechanism for the cholesterol-lowering action of milk PL has not been elucidated.

Objective: To determine whether milk PL can reduce intestinal cholesterol absorption.

Design: Male C57BL/6 mice were fed a high-fat (HF) diet (21% butterfat, 0.15% cholesterol) with or without supplementation of 2.5% (wt/wt) milk PL (n = 10 per group). After 2 weeks, animals were given [^{14}C] cholesterol by intragastric gavage. Intestinal cholesterol uptake was determined by measuring radioactivity in both liver and faeces. Organs were collected for lipid and gene expression analysis.

Result: Dietary PL-supplementation resulted in a significant decrease in hepatic accumulation of intestinally-derived cholesterol (33%, $P < 0.01$). Faecal cholesterol output was increased by 35% in PL-supplemented group compared to the high-fat-fed only group ($P < 0.01$). These changes were associated with increased expression of ABCG5 and ABCG8 in the intestine. The expression of NPC1L1 was not reduced by milk PL supplementation.

Conclusion: The cholesterol-lowering action of milk PL is associated with a significant reduction in intestinal cholesterol uptake and an increased level of faecal cholesterol output. These data suggest that milk PL can exert a primary effect on cholesterol metabolism at the intestinal level.

Poster Session

LIPOPROTEIN(A) ACCELERATES ATHEROSCLEROSIS IN UREMIC MICE

Tanja Xenia Pedersen[1,2], S.P. McCormick[3], S. Tsimikas[4], S. Bro[5], L.B. Nielsen[1,2]

[1]Dept. of Biomedical Sciences, University of Copenhagen, [2]Dept. of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [3]Dept. of Biochemistry, University of Otago, Otago, New Zealand, [4]Division of Cardiovascular Diseases, University of California, San Diego, CA, USA, [5]Dept. of Nephrology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Background: Uremic patients have markedly elevated risk of cardiovascular disease and plasma lipoprotein(a) [Lp(a)] is increased in uremic patients. Lp(a) is a subfraction of apolipoproteinB-100 (apoB) containing lipoproteins, where apolipoprotein (a) (apo(a)) is bound to apoB by a disulfide bond. Lp(a) binds oxidized phospholipids (OxPL) and uremia causes increased formation of OxPL. Thus, Lp(a) may be particularly atherogenic in a uremic setting and we therefore investigated whether transgenic (Tg) expression of human Lp(a) increases atherosclerosis in uremic mice.

Methods: Moderate uremia was induced by 5/6 nephrectomy in Tg mice with expression of human apo(a) (n=19), human apoB-100 (n=18), or human apo(a)+human apoB [Lp(a)] (n=20) and in littermate wildtype (wt) controls (n=15). The uremic mice received a high-fat diet without cholic acid and aortic atherosclerosis was examined 35 weeks later.

Results: LDL-cholesterol was modestly increased to the same extent in apoB-Tg and Lp(a)-Tg mice but normal in apo(a)-Tg and wt mice. Uremia did not result in increased apo(a) or Lp(a) levels. The mean atherosclerotic plaque area in the aortic root was increased 1.8-fold in apo(a)-Tg ($p=0.025$) and 3.3-fold ($p=0.0001$) in Lp(a)-Tg mice compared with apoB-Tg and wt mice. EO6-detectable OxPL were associated with both apo(a) and Lp(a) particles.

Conclusion: Uremia in apo(a)-Tg and Lp(a)-Tg mice expressing a relatively small apo(a) isoform does not result in increased apo(a) or Lp(a) levels. However, apo(a)-Tg and Lp(a)-Tg uremic mice develop increased atherosclerosis compared to apoB and wt mice. OxPL on apo(a) and Lp(a) may contribute to the increased atherogenic potential of these mice.

Poster Session

FENOFIBRATE TREATMENT MODULATES GENE EXPRESSION ACTIVITY IN PERIPHERAL BLOOD MONOCYTES OF HEALTHY VOLUNTEERS

Christelle Foucher[1], A. Menicacci[1], S. Vachenc[1], B. Gerstmayer[2], G. Henrion[1], U. Janssen[2], D. Wilbraham[3], K. Le Malicot[1], J.-C. Ansquer[1]

[1]Solvay Pharmaceuticals, Daix, France, [2]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, [3]Quintiles Limited, London, UK

Objective: The effect of fenofibrate treatment on changes of gene expression in peripheral blood monocytes of healthy subjects was evaluated in a monocentric, open label study.

Methods: Twenty six subjects were treated for 6 weeks with fenofibrate treatment (145 mg/day). CD14+ monocytes were isolated from peripheral blood using a standardized automated immunomagnetic cell separation (autoMACS™ Pro Separator) for microarray analysis using Agilent Whole Human Genome Oligo Microarrays 4x44K (one color), before and after 1 and 6 weeks of treatment. The microarray image files were processed with the Agilent Feature Extraction Software. Differentially expressed genes (DEG) were analyzed using the Rosetta Resolver gene expression data analysis system. A global effect of the treatment was determined using a 2-way ANOVA considering subjects and visits, adjusted by using the Benjamini-Hochberg false-discovery rate method. Pairwise comparisons of DEG between study visits were performed using Newman-Keuls test. A significant effect of the treatment on the genes was concluded for $p \leq 0.01$.

Results: Out of the 7349 sequences referring to 4949 DEG identified with Ingenuity Pathway Analysis, 147 were significantly regulated by fenofibrate. After 1 and 6 weeks of treatment, 54 and 20 DEG were significantly regulated by fenofibrate. Some of these DEG are genes involved in inflammatory, adhesion or lipid metabolism pathways.

Conclusions: Differential gene expression in monocytes was observed early after fenofibrate treatment in healthy subjects. Functional analysis is needed to identify significant biological functions and pathways modulated by fenofibrate.

Poster Session

NON HIGH-DENSITY LIPOPROTEIN CHOLESTEROL IS BETTER PREDICTOR FOR CORONARY RISK, AND IS USEFUL TO EVALUATE THE EFFICACY OF STATIN THERAPY

Kyoichi Mizuno[1], H. Nakamura[2], MEGA Study Group

[1]Nippon Medical School, [2]Mitsukoshi Health and Welfare Foundation, Tokyo, Japan

Aims: To assess the predictability of LDL and HDL related parameters for coronary risk and efficacy of statin therapy using MEGA Study data.

Methods: In the MEGA Study, the 7,832 patients were randomized to diet or diet plus pravastatin and followed up more than 5-years. The 3,966 patients in the diet group were divided into quartiles based on baseline LDL, LDL/HDL ratio, and NHDL level. The coronary risk for each quartile was compared. Further, to compare the predictability, the C-statistics (weighted averaged area under the curves) estimated by the time-dependent ROC curves were estimated. In addition, relationship between on-treatment lipid parameter and coronary risk was also evaluated.

Results: The hazard ratios (HR) for CHD based on the LDL level were 0.93, 1.75, 1.52 in quartile 2, 3, and 4, respectively, compared to quartile 1 (p=0.12). For the LDL/HDL ratio and NHDL, the HR were 1.98, 3.16, and 3.09 (p=0.015), and 1.43, 2.48, 2.75, respectively (p=0.013). The C-statistics were 75.1, 75.6, and 75.7% for LDL, LDL-C/HDL, and NHDL, respectively, indicating that NHDL can predict future CHD more accurately than the other parameters. The HR for NHDL against the diet group for CHD in the diet plus pravastatin group were 0.95 in tertile 1 (160.9-232.2 mg/dL), 0.57 in tertile 2 (145.2-160.8 mg/dL), and 0.59 in tertile 3 (73.6-145.1 mg/dL).

Conclusion: NHDL is a better predictor for future CHD, and an on-treatment NHDL target level of less than 160 mg/dL is appropriate in a population with a 10-year coronary risk < 5%.

Poster Session

THE "BEYOND CHOLESTEROL-LOWERING ACTIVITY" OF PRAVASTATIN. ANALYSIS OF THREE OVERLAPPING DOSE GROUPS

Haruo Nakamura[1], K. Mizuno[2], MEGA Study Group

[1]Mitsukoshi Health and Welfare Foundation, [2]Nippon Medical School, Tokyo, Japan

Objective: To assess the "beyond cholesterol-lowering activity" of pravastatin, post hoc analysis was performed of the results of the primary prevention trial among Japanese with mild or moderate hypercholesterolemia (MEGA Study).

Methods: Of the 7,832 patients randomized to diet alone or diet with pravastatin (10-20 mg/day, average 8.3 mg/day), we analyzed data of 4,845 patients whose on-treatment LDL-C was between 125-164 mg/dL. Patients were classified into 3 groups, diet alone, low-dose pravastatin (< 8.3 mg/day) and high-dose pravastatin (>8.3 mg/day). Average lipid levels and incidence of cardiovascular events were compared between the 3 groups by Cox proportional hazard model.

Results: In the 3 groups, patients who had a cardiovascular event during follow-up had relatively high levels of blood pressure, triglycerides, non-HDL-C, and relatively low HDL-C; a high proportion had diabetes. The CHD event rate was reduced by 32% (p=0.34) in the low-dose group and by 11% (p=0.68) in the high-dose group compared to the diet alone group. The stroke event rate was reduced by 87% (p=0.046) and 30% (p=0.30) respectively in the low- and high-dose groups. All cardiovascular events were reduced by 47% (p=0.059) in low-dose group and 32% (p=0.22) in high-dose group.

Mortality was increased by 47% (p=0.19) in low-dose group and was reduced by 63% (p=0.007) in high-dose group.

Conclusion: Pravastatin has an effect beyond lowering cholesterol as shown by the reduction in CHD events, strokes, and all cardiovascular events, even at the low-dose. To prevent fatal events, the higher dose may be more effective.

Poster Session

EFFICACY OF EZETIMIBE ADDED TO ATORVASTATIN VS. UPTITRATION OF ATORVASTATIN IN SUBGROUPS OF PATIENTS 65-74 YEARS AND ≥75 YEARS OLD

Ori Ben-Yehuda[1], N. Wenger[2], C. Constance[3], F. Zieve[4], J. Lin[5], M. Hanson[5], A. Shah[5], C. Jones-Burton[5], A. Tershakovec[5]

[1]UCSD Medical Center, San Diego, CA, [2]School of Medicine, Emory Univesity, Atlanta, GA, USA, [3]Hopital Maisonneuve-Rosemont Recherche Clinique/Polyclinique, Montreal, QC, Canada, [4]McGuire VA Medical Center, Richmond, VA, [5]Merck & Co., Inc., North Wales, PA, USA

Introduction: Few studies have focused on the efficacy of lipid-lowering therapies in older patients.

Methods: Patients ≥65 years with and without atherosclerotic vascular disease not at LDL-C< 70 or < 100mg/dL, respectively, were randomized to ezetimibe 10mg (E) added to atorvastatin (A)10mg for 12 weeks vs uptitration to A20mg for 6 weeks followed by A40mg for another 6 weeks. Percent change in LDL-C and % patients achieving prespecified LDL-C level after 6 and 12 weeks of A+E vs uptitration of A was assessed in patients 65-74 years (n=812) and ≥75 years (n=218).

Results: After 6 wks, A+E produced greater favorable changes in most lipids vs uptitration of A to 20mg in both age groups. After uptitration to A40mg for 6 weeks, A+E produced greater favorable changes in most lipids in the younger age group, and numerically greater changes in the older age group. A significantly greater proportion of patients achieved LDL-C targets with A+E vs A in both age groups at 6 wks (65-74 yrs: 53% vs 22%; ≥75 yrs: 55% vs 34%) and in patients 65-74 years at 12 weeks (51% vs. 35%), but not in those ≥75 years (A+E: 45% vs A40: 56%) at 12 weeks. Both treatments were generally well-tolerated.

Between treatment difference [A+E minus A] Least Squares Mean % change (95 % CI)	LDL-C, mg/dL; 65-74 years	LDL-C, mg/dL; ≥75 years	Apo B, mg/dL; 65-74 years	Apo B, mg/dL; ≥75 years`	Total C, mg/dL; 65-74 years	Total C, mg/dL; ≥75 years	HDL-C, mg/dL; 65-74 years	HDL-C, mg/dL; ≥75 years
Week 6	-13.6 (-16.0, -11.2)	-14.5 (-19.1, -9.8)	-8.6 (-10.7, -6.6)	-11.0 (-15.0, -7.0)	-7.8 (-9.4, -6.2)	-8.4 (-11.5, -5.3)	1.3 (-0.5, 3.1)	4.2 (0.7, 7.6)
Week 12	-5.7 (-8.8, -2.6)	-0.4 (-6.5, 5.7)	-3.3 (-5.9, -0.7)	-2.8 (-7.7, 2.2)	-2.3 (-4.3, -0.3)	-0.8 (-4.7, 3.1)	3.0 (1.1, 4.8)	3.8 (0.2, 7.3)

[Treatment differences in lipid levels]

Conclusions: E+A generally produced greater favorable changes in most lipid levels vs uptitrating A in patients >65 years.

Poster Session

LONG-TERM STATIN THERAPY IS ASSOCIATED WITH BETTER EPISODIC MEMORY IN AGED FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS IN COMPARISON WITH POPULATION CONTROLS

Laura Hyttinen[1], A. Tuulio-Henriksson[2], A.F. Vuorio[3], N. Kuosmanen[2], T. Härkänen[2], S. Koskinen[2], T.E. Strandberg[4,5]

[1]Department of Medicine, North Karelia Central Hospital, Joensuu, [2]National Institute for Health and Welfare, [3]Department of Medicine, University of Helsinki, Helsinki, [4]Institute of Health Sciences/Geriatrics, [5]Unit of General Practice, University of Oulu, Oulu, Finland

Aims: To compare the cognitive status of aged familial hypercholesterolemia (FH) patients treated with long-term statin therapy with that of population controls.

Methods: A comprehensive cohort of 43 elderly (age ≥ 65 years) FH patients living in eastern Finland was identified, 37 of whom (aged 65 to 84 years) agreed to participate. All but one of these FH patients had been using statins for approximately 15 years. Population-based controls (aged 65 to 84 years, n=309) were the participants of the Health 2000 Survey living in eastern Finland. The cognitive assessment was conducted with tests for verbal fluency, Word List Learning (WLL) and Word List Delayed Recall (WLDR) subtests in the CERAD (Consortium to Establish a Registry for Alzheimer's disease) test battery.

Results: After adjustment for age, sex, education, diabetes mellitus and coronary heart disease, FH patients were more likely to be in the top tertile of the WLDR (OR 3.40, 95% confidence interval [CI] 1.52 - 7.63) and WLL3 (2.83, 95% CI 1.28 - 6.25) subtests. When the FH patients were subdivided according to the median length of their statin therapy, the ORs to be in the top tertile in the WLDR subtest were 1.65 (95% CI 0.52 - 5.25) for those with less and 5.50 (95% CI 1.74 - 17.72) in those individuals with more than median length of statin therapy.

Conclusions: Aged FH patients receiving long-term statin therapy exhibited better episodic memory than population controls, and this association became even more pronounced with longer statin therapy.

Poster Session

ACTIVATION OF LYSOSOMES BY INTRACELLULAR TRIACYLGLYCEROL ACCUMULATION CONTRIBUTES TO THE DEVELOPMENT OF HEPATIC INSULIN RESISTANCE AND OXIDATIVE STRESS

Monika Cahova[1], Z. Drahota[2], H. Dankova[1], O. Oliyarnyk[1], E. Palenickova[1], Z. Papackova[1], I. Markova[1], L. Kazdova[1]

[1]Department of Metabolism and Diabetes, Institute for Clinical and Experimental Medicine, [2]Institute of Physiology, Prague, Czech Republic

Aims: To test the hypothesis that dysregulation of endogenous triacylglycerol (TAG) degradation in fatty liver is associated with development of hepatic insulin resistance (HIR) and contributes to mitochondrial dysfunction and oxidative stress.

Methods: Liver TAG accumulation was induced by 2-week high-fat diet (HFD) administration to male Wistar rats (300±15 g).

Results: We identified lysosomal lipase (LAL) as the only lipase capable to degrade intracellular TAG. On standard diet, LAL activity was up-regulated in fasting and down-regulated in fed state. LAL mRNA expression followed similar pattern. Increase in LAL activity was associated with enzyme translocation into phagolysosome (PhL) fraction. HFD increased LAL activity in fed state. HFD did not affect LAL mRNA transcription or total LAL protein expression but stimulated the translocation of LAL protein into PhL fraction. Enhanced TAG degradation in lysosomes was associated with intensified autophagy (higher LC3-II expression). HFD feeding resulted in enhanced production of diacylglycerol, impaired insulin signalling in liver and activation of PKCε. In HFD group we proved increased PhL fragility together with higher lipoperoxide production and cytochrome c release into cytosol. Application of lysosome stabilizer (dexamethasone) partly ameliorated these symptoms.

Conclusion: The LAL-mediated lipolysis of intracellular TAG is increased in fatty liver. The overproduction of diacylglycerol may represent the causal link between steatosis and HIR via PKCε activation. Activated phagolysosomes may contribute to the alterations in mitochondrial function via

1) supplying excess of fatty acids for oxidation and

2) deterioration of mitochondrial membrane by released lysosomal enzymes, both resulting in increased ROS production.

Poster Session

ARE POST-TREATMENT LOW DENSITY LIPOPROTEIN SUBCLASS PATTERN ANALYSES POTENTIALLY MISLEADING?

Harold Bays[1], S. Conard[2], L.A. Leiter[3], E. Jensen[4], S. Bird[4], M.E. Hanson[4], A.M. Tershakovec[4]

[1]Louisville Metabolic and Atherosclerosis Research Center, Louisville, KY, [2]University of Texas Southwestern Medical School, Dallas, TX, USA, [3]St Michael's Hospital and University of Toronto, Toronto, ON, Canada, [4]Merck & Co., Inc., North Wales, PA, USA

Introduction: Therapies that lower LDL-C often increase small, LDL particles (Pattern B), which may be misinterpreted as a worsening of atherosclerotic coronary heart disease (CHD) risk.

Methods: Hypercholesterolemic patients at high CHD risk were stabilized on atorvastatin (AT) 40 mg followed by randomization to ezetimibe (EZ) 10 mg + AT40 mg vs doubling of AT to 80 mg for 6 weeks. Vertical Auto Profile-II analysis was performed on the overall population, as well as on those grouped by baseline triglyceride (TG) level < 150 mg/dL or ≥150 mg/dL.

Results: In the full data set and in the higher/lower TG subsets, the proportion of Pattern B increased with both treatments. Both EZ+AT and AT reduced LDL-C levels and decreased the cholesterol content of most LDL subfractions (LDL_{1-4}). Together, the cholesterol content in the larger LDL_1 and LDL_2 subfractions was generally reduced more than in the smaller LDL_3 and LDL_4 subfractions together. Both treatments reduced apo B, reflecting a reduction in overall atherogenic lipoprotein particle number.

	mg/dL (SD)	Pattern B (%)	LDL1	LDL2	LDL3	LDL4	LDL-C	Apo B
A80 mg (N=222)	Baseline	128/222 (57.7)	14.0 (6.5)	16.5 (12.1)	41.5 (13.0)	9.0 (7.4)	89.7 (16.0)	102.0 (18.5)
	Week 6	141/222 (63.5)	12.0 (4.7)	15.0 (10.2)	37.0 (13.0)	9.0 (7.4)	79.1 (19.9)	93.2 (20.0)
A40 mg + EZ (N=225)	Baseline	127/225 (56.4)	14.0 (4.7)	17.0 (14.9)	38.0 (14.0)	9.0 (7.4)	88.6 (16.3)	101.1 (18.8)
	Week 6	147/225 (65.3)	10.0 (3.7)	13.0 (7.4)	29.0 (11.2)	8.0 (4.7)	64.1 (19.9)	82.5 (19.3)

[Baseline and Study-end Values]

Conclusion: LDL-C lowering therapy may disproportionately reduce larger (more easily cleared) LDL particles, and thus increase Pattern B. A post-treatment change to Pattern B alone may be misleading in assessing the efficacy of cholesterol lowering therapy.

Poster Session

EZETIMIBE/SIMVASTATIN 10/20 MG VERSUS ROSUVASTATIN 10 MG IN HIGH RISK HYPERCHOLESTEROLEMIC PATIENTS STRATIFIED BY PRIOR STATIN TREATMENT POTENCY

M. Viigimaa[1], H. Vaverkova[2], M. Farnier[3], M. Averna[4], L. Missault[5], Q. Dong[6], A. Shah[6], M. Hanson[6], **P Brudi**[6]

[1]Tallinn University of Technology North-Estonia Regional Hospital, Tallinn, Estonia, [2]Internal Medicine, III Interni Klinika FN Olomouc, Olomouc, Czech Republic, [3]Point Medical Rond Point de la Nation, Dijon, France, [4]Dipartimento di Medicina Clinica e Patologie Emergenti, Paolo Giaccone Universita di Palermo, Palermo, Italy, [5]Department of Cardiology, St Jan Hospital, Bruge, Belgium, [6]Merck & Co., Inc., North Wales, PA, USA

Objective: This *post-hoc* analysis compared the lipid-altering efficacy of Ezetimibe/Simvastatin 10/20 mg (EZ/Simva) versus Rosuvastatin 10 mg (Rosuva) in patients stratified by statin potency/dose prior to randomization.

Methods: Patients with elevated low-density lipoprotein cholesterol (LDL-C) despite prior statin treatment (n=618) were randomized 1:1 to EZ/Simva 10/20mg or Rosuva 10mg for 6 weeks. Percent change from baseline in lipids and attainment of lipid targets were assessed within each subgroup (low potency n=369, high potency n=249). Consistency of the treatment effect across subgroups was evaluated by testing for treatment-by-subgroup interaction. No multiplicity adjustments were made.

Results: Significant treatment-by-subgroup interaction occurred for LDL-C (p=0.013), total cholesterol (p=0.025), non-HDL-C (p=0.032), and Apolipoprotein-B (p=0.016) with greater between treatment differences in favor of EZ/Simva observed in patients from the high potency stratum vs low potency stratum. Individual and triple target attainment was higher for Eze/Simva compared with Rosuva in both strata.

Least squares mean % change from baseline, mg/dL(SE)	LDL-C	Total C	Apo B	Non-HDL-C	HDL-C	% attaining LDL-C<100 mg/dL (SE)	% attaining non-HDL-C<130 mg/dL (SE)	% attaining Apo-B<90 mg/dL (SE)	% attaining LDL-C<100 and non-HDL-C<130 and Apo-B<90 mg/dL (SE)
Low potency prior statin/ Rosuva 10 mg	-24.2 (1.6)	-15.4 (1.2)	-15.4 (1.5)	-20.9 (1.6)	4.1 (1.3)	80% (2.9)	47% (3.7)	47% (3.7)	45% (3.75)
Low potency prior statin/ EZ/Simva 10/20	-31.5 (1.6)	-20.0 (1.2)	-20.4 (1.4)	-27.3 (1.5)	3.5 (1.2)	70% (3.4)	49% (3.8)	49% (3.8)	49% (3.73)
High potency prior statin/ Rosuva 10 mg	-8.1 (2.8)	-4.4 (1.8)	-0.9 (2.1)	-6.3 (2.4)	3.3 (1.6)	61% (4.4)	15% (3.3)	15% (3.3)	15% (3.26)

High potency prior statin/ EZ/Simva 10/20	-24.6 (2.7)	-15.1 (1.8)	-14.7 (2.0)	-20.2 (2.4)	2.4 (1.6)	36% (4.4)	32% (4.3)	32% (4.3)	31% (4.19)

[Lipid changes and goal attainment]

Conclusions: Compared with Rosuva, switching to EZ/Simva provided greater reductions in LDL-C, total cholesterol, non-HDL-C and Apolipoprotein-B and higher lipid target attainment in patients on prior statin treatment, regardless of potency, although patients treated with higher potency statins prior to randomization resulted in greater between treatment differences in favor of EZ/Simva.

Poster Session

PIOGLITAZONE SUPPRESSES ONLY PLASMA SMALL, DENSE LDL BUT NOT LARGE LDL IN TYPE 2 DIABETIC PATIENTS

Masahiko Miyagi[1], K. Kuboki[1], T. Matsumoto[1], R. Iga[1], N. Masai[1], K. Iso[1], E. Murakami[2], S. Kodate[3], T. Hirano[3], G. Yoshino[1]

[1]Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine (Omori), Toho University School of Medicine, [2]Clinical Laboratory, Toho University Medical Center Omori Hospital, [3]Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan

Aims: To examine the effect of pioglitazone on plasma atherogenic low-density lipoprotein (LDL), especially small, dense LDL-cholesterol (sLDL-C) and large LDL-C (lLDL-C) separately, in type 2 diabetic patients.

Methods: Forty Japanese patients (15 male and 25 female) were enrolled. Pioglitazone add-on treatment was started with 7.5 or 15 mg once daily for 3 months. Blood samples were collected after an overnight fast at baseline and after 3 months. Plasma sLDL-C was measured by the magnesium-precipitation method. lLDL-C was calculated by subtracting sLDL-C from total LDL-C.

Results: Fasting plasma glucose (FPG), glycohaemoglobin (HbA_{1C}), triglyceride (TG), and sLDL-C levels were significantly suppressed ($p < 0.05$), while total cholesterol (TC), LDL-C, high-density lipoprotein-cholesterol (HDL-C), remnant-like particle-cholesterol (RLP-C), and apolipoprotein B (apoB) levels showed no significant changes. Interestingly, no significant change was detected in plasma lLDL-C. Even when the subjects were divided into two groups according to the percent decrease in HbA_{1C} level: responders (more than 5% decrease; 23 subjects) and non-responders (within one; 17 subjects), sLDL-C levels were significantly decreased in each group (-31.6 and -28.3% respectively, $p < 0.05$).

parameters		All subjects (n=40)	Responders (n=23)	Non-responders (n=17)
HbA1c (%)	baseline	7.1 ± 1.0	7.2 ± 0.8	6.9 ± 1.3
	3 months	6.7 ± 1.0*	6.5 ± 0.7**	6.9 ± 1.3
sLDL-C (mg/dl)	baseline	36.5 ± 19	32.4 ± 14	42.0 ± 24
	3 months	25.6 ± 12**	22.2 ± 10**	30.1 ± 14*
lLDL-C (mg/dl)	baseline	87 ± 26	91 ± 26	81 ± 25
	3 months	93 ± 22	94 ± 21	92 ± 24
All values are represent as Mean ± SD. *: $p < 0.05$ ve baseline, **: $p < 0.01$ vs baseline				

[Comparison between baseline and after treatment]

Conclusions: These results suggest that pioglitazone decreased only small-sized LDL, but not large-sized LDL. This effect was independent of improving glycaemic control. It is speculated that hepatic production of large-sized (TG-rich) very low-density lipoprotein (VLDL), a precursor of small, dense LDL, was suppressed through the amelioration of TG metabolism by pioglitazone. In contrast, a production of normal-sized VLDL, a precursor of large LDL, seems to be unaffected by pioglitazone.

Poster Session

DOES GENDER IMPACT ON STATIN DOSE RESPONSE? RESULTS FROM THE VOYAGER INDIVIDUAL PATIENT DATA META-ANALYSIS

Pia Lundman[1], S.J. Nicholls[2], G. Brandrup-Wognsen[3], M. Palmer[4], P.J. Barter[5]

[1]Danderyd Hospital, Karolinska Institute, Stockholm, Sweden, [2]Cleveland Clinic Foundation, Cardiovascular Medicine, Cleveland, OH, USA, [3]AstraZeneca, Mölndal, Sweden, [4]Keele University, Keele, UK, [5]Heart Research Institute, Sydney, NSW, Australia

Background: Despite unequivocal cardiovascular benefit in large clinical trials, the relative benefit of statin therapy in women remains uncertain. This study investigated the impact of gender on lipid responses to increasing statin doses.

Methods: The relationships between rosuvastatin (R; n=18897) or atorvastatin (A; n=15141) dose and changes in lipid parameters were determined in patients (43% female) in the VOYAGER individual patient data meta-analysis of 37 randomised, comparative studies, including 32258 patients overall.

Results: A relationship was observed between increasing statin dose and improvements in atherogenic lipid levels in both genders (Table), with women having better LDL-C and nonHDL-C lowering but a comparable HDL-C raising *versus* men.

% change[#]	R 5 mg	R 10 mg	R 20 mg	R 40 mg	A 10 mg	A 20 mg	A 40 mg	A 80 mg
Men, n	332	6516	2141	1802	4484	2316	829	1389
LDL-C	-38.4	-42.9	-49.2	-53.9	-34.4	-40.9	-45.6	-50.1
NonHDL-C	-35.2	-39.1	-44.7	-49.3	-31.9	-37.8	-41.8	-46.5
HDL-C	5.5	6.8	7.2	8.6	5.1	4.5†	3.2	2.7
Women, n	338	5174	1413	1181	3353	1592	495	683
LDL-C	-40.1	-46.1*	-50.7*	-55.6*	-37.3*	-42.9*	-46.4	-49.9
NonHDL-C	-36.1	-41.9*	-46.1*	-50.6*	-34.3*	-39.2*	-42.3	-46.1
HDL-C	6.3	6.3	6.9	8.2	4.7	3.0	2.2	2.6
*p<0.05 vs the same drug dose in men; †p=0.004 vs same drug dose in women; #Least square mean % change in each lipid parameter from baseline								

[Table]

Conclusions: The finding of consistently more beneficial changes in levels of atherogenic lipid parameters with increasing statin doses in women supports their use more effectively to reduce cardiovascular risk.

Poster Session

THE EFFECTS OF EZETIMIBE, ALONE OR COMBINED WITH ORLISTAT, ON TRIGLYCERIDE-RICH LIPOPROTEIN METABOLISM IN OVERWEIGHT AND OBESE PATIENTS WITH HYPERLIPIDAEMIA

E. Nakou[1], T. Filippatos[1], C. Kostara[2], A. Agouridis[1], E. Bairaktari[2], **Moses Elisaf**[1]

[1]Department of Internal Medicine, Medical School, [2]Laboratory of Clinical Chemistry, Medical School, University of Ioannina, Ioannina, Greece

Background: Ezetimibe treatment in most trials significantly reduced plasma triglyceride (TG) levels.

Objective: We investigated the factors influencing TG reduction during ezetimibe, alone or in combination with orlistat, administration. Moreover, we examined the effect of ezetimibe treatment, alone or combined with orlistat, on apolipoprotein C-II (apoC-II) and C-III (apoC-III) levels.

Methods: Eighty six subjects were prescribed a low-fat low-calorie diet and were randomly allocated to receive ezetimibe 10 mg/day (E group), orlistat 120 mg 3 times daily (O group), or both (OE group) for 6 months. Anthropometric and biochemical parameters were assessed at baseline and after 6 months of treatment. Plasma apoC-II and apoC-III were determined by an immunoturbidimetric assay.

Results: We observed significant in-group reductions in serum TG levels in all treatment groups. Ezetimibe administration and orlistat/ezetimibe combination significantly reduced the concentrations of apoC-II and apoC-III. Multivariate analysis showed that in E group apoC-III reduction and baseline TG levels were independently positively correlated, while baseline apoC-II levels were negatively correlated, with TG lowering. In OE group apoC-III reduction was the only independent contributor to TG reduction.

Conclusions: Ezetimibe-mediated TG reduction is independently positively associated with apoC-III alterations and baseline TG, while it is negatively associated with baseline apoC-II levels. When ezetimibe is combined with orlistat, apoC-III reduction is the only independent contributor to TG alterations.

Poster Session

SAFETY AND EFFICACY OF EZETIMIBE/SIMVASTATIN COMBINATION VERSUS ATORVASTATIN IN PATIENTS 65 YEARS OF AGE AND OLDER

J.M. Foody[1], W.V. Brown[2], F. Zieve[3,4], A.J. Adewale[5], D. Flaim[5], R.S. Lowe[5], C. Jones-Burton[5], A.M. Tershakovec[5]

[1]Harvard Medical School, Brigham and Women's, Boston, MA, [2]Emory University School of Medicine, Atlanta, GA, [3]McGuire VA Medical Center, [4]Virginia Commonwealth University, Richmond, VA, [5]Merck & Co., Inc., North Wales, PA, USA

Background: Over 80% of CHD related mortality occurs in patients ≥65 years old. Few studies have evaluated lipid-lowering strategies specifically in older adults. This study assessed the efficacy and safety of simvastatin/ezetimibe and atorvastatin in hypercholesterolemic patients ≥65 years with or without cardiovascular disease.

Methods: Multicenter, 12-week, randomized, double-blind, parallel-group trial. 1,289 patients randomized to ezetimibe 10mg/simvastatin 20mg (E10/S20) versus atorvastatin 10mg (A10) or A20, and E10/S40 versus A40. Pre-specified efficacy endpoints: percent change from baseline LDL-C, attainment of LDL-C< 1.81 and < 2.59mmol/L, safety/tolerability, percent change from baseline lipids.

Results: Ezetimibe/simvastatin produced significantly greater percent reductions in baseline LDL-C and significantly higher attainment of LDL-C< 1.81 and < 2.59mmol/L than atorvastatin for all dose comparisons. Remaining comparisons (±CVD) favored ezetimibe/simvastatin (Table). Non-HDL-C, total-C, and apolipoprotein-B improvements were significantly greater with ezetimibe/simvastatin than atorvastatin for all comparisons. HDL-C and triglyceride results were variable. All treatments were well tolerated.

Conclusion: Ezetimibe/simvastatin provided significantly greater improvements in key lipid parameters and higher attainment of LDL-C targets than atorvastatin with comparable tolerability, and remains a useful treatment option for dyslipidemia in older patients.

LDL-C Endpoints (*p<0.5, **p<0.01, ***p<0.001 for specified dose comparisons)	**A10 (N=242)**	**E10/S20 (N=232)**	**A20 (N=238)**	**E10/S40 (N=236)**	**A40 (N=239)**
LDL-C Baseline mean - mmol/L (% Change from Baseline)	4.33 (-39.5)***	4.30 (-54.2)	4.27 (-46.6)***	4.24 (-59.1)	4.37 (-50.8)***
	Number of Patients Achieving Pre-Specified LDL-C (%)				
LDL-C <1.81 mmol/L (all patients)	24 (9.9)***	119 (51.3)	62 (26.1)***	161 (68.2)	91 (38.1)***
LDL-C <1.81 mmol/L (with CVD)	7 (10.0)***	36 (44.4)	25 (31.6)	52 (65.8)	32 (44.4)*
LDL-C <2.59 mmol/L (all patients)	142(58.7)***	194 (83.6)	183 (76.9)*	213 (90.3)	190 (79.5)**
LDL-C <2.59 mmol/L (without CVD)	102 (59.3)**	124 (82.1)	121 (76.1)	141 (90.4)	135 (80.8)

[% Change in LDL - Attainment of LDL Targets]

Poster Session

THE RELATIONSHIP BETWEEN FIBRINOLYSIS, LIPID PROFILE AND GLUCOSE METABOLISM CHARACTERISTICS IN RANDOMLY SELECTED MOSCOW RESIDENTS AGED 55 AND OVER

Victoria Metelskaya[1], L. Ratnikova[1], S. Shalnova[1], M. Shkolnikova[2], A. Deev[1], V. Shkolnikov[3]

[1]National Research Centre for Preventive Medicine, [2]Federal Center for Cardiac Arrhythmias, Moscow, Russia, [3]Max-Plank Institute for Demography Research, Rostock, Germany

Background: This particular analysis was conducted within the framework of Stress And Health in Russia (SAHR) which is an ongoing prospective population-based cohort study among Moscow residents aged 55+, and aimed to reveal associations between activity of fibrinolysis and characteristics of lipoprotein and glucose metabolism.

Material and methods: The whole sample (n=1863; 52.2% females) was stratified in three groups according to activity of fibrinolysis measured as Euglobulin Clot Lysis Time (ECLT): normofibrinolysis, ECLT 180-260 min, hypofibrinolysis, ECLT>260 min, hyperfibrinolysis, ECLT< 180 min.

Results: Subjects with hypofibrinolysis have 19.2% higher fibrinogen level than with normofibrinolysis, whereas those with hyperfibrinolysis didn't differ from normofibrinolysis. In hypofibrinolysis group elevated TG (by 14.4%) and decreased HDL C (by 6.1%) were found; $p< 0.01$. On the contrary, lipoprotein profile in hyperfibrinolysis subjects was less atherogenic than in normofibrinolysis. Hypofibrinolysis group characterized by slightly increased serum glucose (3%) and insulin (20.5%) while hyperfibrinolysis group characterized by lower levels of glucose (8.2%) and insulin (29.9%) than normofibrinolysis. Thus, significant abnormalities in lipid profile and glucose metabolism in hypofibrinolysis group were found. Moreover, risk of arterial hypertension (AH) was 29% higher than for normofibrinolysis; of type 2 diabetes mellitus (DM) by 41% ($p< 0.05$), while subjects with hyperfibrinolysis have lower risk of AH by 53%, and of DM by 58% than those with normofibrinolysis ($p< 0.05$).

Conclusion: Disturbed hemostasis was associated with abnormal shifts in lipoproteins and markers of glucose utilization.

Acknowledgements: The SAHR Study was supported by research grant No.R01AG026786 from the National Institute of Aging (USA)

Poster Session

INFLUENCE OF NOVEL SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS ON COPPER-MEDIATED OXIDATION OF HUMAN LDL

Jens Pietzsch[1], F.-J. Pietzsch[1], M. Laube[2], R. Bergmann[1], F. Wuest[3], J. Steinbach[1,2], T. Kniess[2]

[1]Department of Radiopharmaceutical Biology, [2]Department of PET Tracers, Institute of Radiopharmacy, Research Center Dresden-Rossendorf, Dresden, Germany, [3]Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada

Clinical investigations showed a link between the extended use of rofecoxib, a selective COX-2 inhibitor (coxib), and increased risk for atherothrombotic events. This has led to general concern over the cardiovascular safety of coxibs. Subsequently, rofecoxib has been shown to increase oxidation of LDL and membrane lipids, whereas other coxibs did not, providing a biochemical rationale for differences in cardiovascular risk among coxibs. We investigated the independent effects of eight COX-2 inhibitors (two cyclopentene-/four indole-based sulfone/sulfonamide derivatives) and two non-sulfonyl indomethacin derivatives, which all were synthesized as lead compounds for development of novel COX-2 imaging radiotracers, on both human LDL lipid and protein oxidation, compared to celecoxib and quercetin as controls. The results showed that indole derivatives (50 nM to 1 µM) significantly concentration-dependently increased the *lag*-time for Cu^{2+}-mediated LDL conjugated diene formation (>10%, $p < 0.01$) and, furthermore, significantly decreased formation of specific markers of protein oxidation (>18%, $p < 0.01$) *in vitro*. Addition of indole-based coxibs to human plasma increased the oxygen radical antioxidant capacity (ORAC) by 6-14% ($p < 0.01$). By contrast, other coxibs (cyclopentene and indomethacin derivatives) did not show effects on lipid and protein oxidation and had no significant effect on plasma ORAC values, even at suprapharmacologic concentrations. Additionally, a radiotracer-based analysis showed that indole derivatives interact differently with LDL subfractions and other lipoprotein particles, than the cyclopentene and indomethacin derivatives, respectively, suggesting a physico-chemical basis for the observed anti-oxidant activity. These findings may provide further insights for evaluation of potential cardiovascular risk for selective COX-2 inhibitors.

Poster Session

LDL-APHERESIS SELECTIVELY INDUCES CHANGES IN THE LIPIDOME AND PROTEOME OF APO-AI-CONTAINING LIPOPROTEIN PARTICLES IN FAMILIAL HYPERCHOLESTEROLEMIA

Alexina Orsoni[1,2,3], M. Guérin[1,2,3], M. Couturier[1,4], G.M. Dallinga-Thie[5], J.H.M. Levels[5], S. Saheb[6], M. Atassi-Dumont[6], D. Bonnefont-Rousselot[4,7], A. Kontush[1,2,3], A. Carrié[1,2,3], E. Bruckert[8], M.J. Chapman[1,2,3]

[1]INSERM U939, [2]Université Pierre et Marie Curie-Paris, [3]Hôpital Pitié-Salpêtrière, APHP, [4]Université Descartes Paris 5, Paris, France, [5]Department of Experimental Vascular Medicine, Academic Medical Center Amsterdam, Amsterdam, The Netherlands, [6]Haemobiotherapy Unit, [7]Department of Biochemistry, [8]Department of Endocrinology, Hôpital Pitié-Salpêtrière, APHP, Paris, France

Objective: By electrostatic and non-ionic interactions, LDL-apheresis efficiently reduces elevated plasma levels of atherogenic apoB-containing lipoproteins (-75%; VLDL, LDL, Lp(a)) in FH; however, plasma proteins and HDL (-20%) are removed. Does LDL-apheresis in FH induce selective changes in apoAI-containing particle profile, with concomitant changes in lipidome and proteome and potential impact on atherogenesis?

Methods and results: Analyses of the lipidome and proteome of plasma lipoprotein subspecies fractionated by isopycnic density gradient ultracentrifugation were conducted immediately pre- and post-LDL-apheresis. Additionally, the activities of intravascular lipoprotein remodelling factors (CETP, LPL, HL, LCAT) were evaluated. Post-LDL-apheresis, LpAI:AII particles were preferentially depleted (-26%); likewise, apoE-containing HDL, distributed as HDL2b and HDL2a, were removed (-55%), thus apoE:ApoAI ratio in HDL was decreased (-40%). The proportions of the 20:4/16:0 and 22:6/16:0 PC species increased in the lipidome across the HDL particle density spectrum, while those of CE 20:4 and 18:2 fell (-20%). These significant changes were accompanied by (i) marked reduction in plasma CETP activity (-50%) and mass (-24%), without change in LCAT activity (ii) by transient increase in circulating lipase activities mediated by heparin infusion, and (iii) by preferential removal of HDL2 particles rich in CE and apoE; concomitantly, SELDI-TOF analysis indicated loss of apoE, apoAII and ApoCs.

Conclusion: LDL-apheresis mediates selective changes in the lipidome and proteome of HDL particle subspecies in FH, which result from the combined effects of selective particle removal and altered particle remodelling. The net effect of such LDL-apheresis-induced changes on the overall atheroprotective function of HDL remains indeterminate.

Poster Session

CIRCULATING PCSK9 LEVELS ARE ASSOCIATED WITH FRUCTOSE-INDUCED HYPERTRIGLYCERIDAEMIA

Bertrand Cariou[1], M. Le Bras[1], C. Langhi[1], M. Bortolotti[2], K.-A. Lê[2], C. Le May[1], R. Kreis[3], C. Boesch[3], M. Krempf[1], L. Tappy[2], P. Costet[1]

[1]L'Institut du Thorax, INSERM U915, Nantes, France, [2]Department of Physiology, University of Lausanne, Lausanne, [3]Department of Clinical Research, MR Spectroscopy and Methodology, University of Bern, Bern, Switzerland

Introduction: PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) is a secreted proprotein convertase that plays a key role in cholesterol homeostasis by decreasing LDL receptor protein expression.

Objective: We aim to assess the nutritional regulation of circulating PCSK9 in healthy young subjects.

Design: Healthy volunteers (6-15/group) were subjected to various nutritional intervention protocols, including:

i) acute post-prandial hyperlipidemic challenge,

ii) 4 days high-fat (HF) or high-fat/high-protein (HFHP) diets,

iii) 7 days high-fructose (HFruc) or high-glucose (HGluc) diets.

Protocols were performed in a crossover design with an isocaloric diet as control. Plasma PCSK9 concentrations were measured by an ELISA assay. Intrahepatocellular (IHCL) and intramyocellular (IMCL) lipids were measured by ^{1}H-RMN spectroscopy. Hepatic and whole-body insulin sensitivity were assessed with a two-step hyperinsulinemic-euglycemic clamp (0.3 and 1.0 mU.kg^{-1}.min^{-1}).

Results: Circulating PCSK9 levels were not altered in response to HF or HFHP, as well as following a hyperlipidemic oral test. PCSK9 was significantly increased by 28% (p=0.04) and 47% (p=0.02) following HFruc and HGluc diets, respectively. The fructose-induced PCSK9 increase was more pronounced in healthy off-spring of type 2 diabetic patients (+34%, p=0.001). Spearman's correlations revealed that plasma PCSK9 concentrations upon HFruc were positively associated with plasma VLDL and IHCL, and inversely correlated with both hepatic (hepatic glucose production) and whole-body (glucose disposal rate) insulin sensitivity.

Conclusions: Circulating PCSK9 rise upon high-carbohydrate diet and correlate with hepatic steatosis and VLDL concentrations, reinforcing the hypothesis for a potential role of PCSK9 in triglyceride metabolism.

Poster Session

ABCG5/G8 DEFICIENCY INCREASES TRIGLYCERIDE LEVELS BY AFFECTING MULTIPLE METABOLIC PATHWAYS

Jesús Méndez-González[1,2], J. Julve[1,3], N. Rotllan[1,3], F. Blanco-Vaca[1,2,3], J.C. Escolà-Gil[1,3]

[1]Servei de Bioquímica and Institut de Recerca, Hospital de la Santa Creu i Sant Pau, [2]Departament de Bioquímica i Biologia Molecular, Universitat Autònoma, [3]CIBER de Diabetes y Enfermedades Metabólicas Asociadas, CIBERDEM, Barcelona, Spain

Introduction and objective: Mutations in ABCG5 or ABCG8 transporters are responsible for sitosterolemia, an autosomal recessive disease characterized by accumulation of plant sterols. These plant compounds can act as LXR agonists and elevate plasma triglycerides (TG). However, the effects of ABCG5/G8 deficiency on TG metabolism remain unknown. The objective of the study was to examine TG metabolism in a mouse model of sitosterolemia.

Methods and results: ABCG5/G8$^{(+/+,+/-,-/-)}$ mice fed with a chow diet were used. Plasma TG were 2.6 and 4.3-fold higher in fasted ABCG5/G8$^{(+/-)}$ and ABCG5/G8$^{(-/-)}$ mice than in wild-type animals, respectively. These changes were a consequence of alterations in multiple metabolic pathways: first, a dose-dependent increase in liver LXR, FAS and ACAC gene expression was observed; second, liver TG production was 1.3-fold higher in ABCG5/G8$^{(-/-)}$ and ABCG5/G8$^{(+/-)}$ than in wild-type mice; third, postheparin plasma LPL activities were significatively lower in ABCG5/G8$^{(+/-)}$ (1.8-fold) and ABCG5/G8$^{(-/-)}$ mice (5.4-fold) than in wild-type; and finally and unexpectedly, TG intestinal secretion, determined after an oral fat gavage of glycerol tri[9,10(n)-^{3}H] oleate, was 5.8-fold higher in ABCG5/G8$^{(-/-)}$ than in wild-type mice.

Conclusions: ABCG5/G8 deficiency markedly increases TG levels by altering liver and intestinal TG secretion and impairing TG catabolism.

Poster Session

THE ROLE OF SNARE PROTEINS IN HUMAN INSULIN RESISTANCE

Pontus Bostrom[1], L. Andersson[1], B. Vind[2], L. Haversen[1], Y. Wickstrom[1], E. Larsson[1], P.-A. Jansson[1], M. Svensson[1], R. Branemark[3], C. Ling[4], H. Beck-Nielsen[2], J. Boren[1], K. Hojlund[2], S.-O. Olofsson[1]

[1]Wallenberg Laboratory for Cardiovascular Research and Center for Cardiovascular and Metabolic Research; Physiology Department, Sahlgrenska University Hospital, Gothenburg, Sweden, [2]Diabetes Research Centre, Department of Endocrinology, Odense University Hospital, Odense, Denmark, [3]Department of Orthopedic Surgery, Sahlgrenska University Hospital, [4]Department of Clinical Sciences CRC, University Hospital of Malmö, Gothenburg, Sweden

Insulin resistance and type II diabetes are major risk factors for atherogenesis and cardiovascular disease, and is closely associated with neutral lipid accumulation in non-adipocytes such as skeletal muscle. In a previous report, we presented a novel mechanism, linking lipid storage with insulin resistance via the SNARE protein SNAP23. In more detail, SNAP23 was diverted from the GLUT4 fusion machinery towards lipid droplets upon induction of lipid storage, causing insulin resistance - an effect which could be completely reversed by over expression of SNAP23 (Bostrom et. al. 2007).

In the present study, we have continued these studies in muscle biopsies from well characterized type II diabetic patients and closely matched lean/obese controls. We have also utilized human, primary myocyte cultures for functional studies in vitro.

We observe a major increase in SNAP23 protein levels in diabetic muscle, mostly localized to cytosolic compartments. In fact, we could demonstrate a close correlation between plasma membrane SNAP23 and CLAMP insulin resistance ($r=0.91$, $p=0.012$), in agreement with our previous results, and suggestive of a major role for SNAP23 in human insulin resistance. We did also observe an up regulation of the SNARE associated protein Munc18c, known to disrupt SNAP23 in the plasma membrane compartment. Munc18c levels correlated with insulin resistance, and over expression of Munc18c in human myocytes caused translocation of SNAP23. Thus, we propose a model where Munc18c is up regulated in diabetes causing missorting of SNAP23 and thereby insulin resistance.

Poster Session

ROLE OF ABCA1 TRANSPORTER IN HUMANS AND RELATIONSHIP TO ATHEROSCLEROSIS

Ekaterina Demina[1], V. Miroshnikova[1], A. Denisenko[2], A. Schwarzman[1]

[1]Petersburg Nuclear Physics Institute of RAS, Gatchina, [2]Institute of Experimental Medicine of the NorthWest Branch of the Russian Academy of Medical Sciences, Saint Petersburg, Russia

Reverse cholesterol transport (RCT) is believed to be a critical mechanism by which high density lipoproteins (HDL) exert a protective effect on the development of atherosclerosis. ABCA1 transporter is known to play important role in RCT from peripheral tissues. However its influence on atherosclerosis development remains not studied completely. According to our hypothesis ABCA1 gene expression level may influence on metabolism of antiatherogenic HDL and contribute to atherosclerosis development. Using real-time quantitative polymerase chain reaction we compared the mRNA levels of ABCA1, and one of its key regulators, the liver X receptor α (LXR α), between banks of cDNA, isolated from leukocytes and macrophages of controls (N=9) and patients with atherosclerosis diagnosed using angiography(N=18). LXR α mRNA levels in macrophages did not differ from the one in leukocytes in the group of patients and in the control group. In the group of patients with atherosclerosis 2-fold increase of ABCA1 gene expression level in macrophages when compared with the one in leukocytes was discovered. Mean levels of ABCA1 mRNA levels in macrophages and leukocytes of patients are 1.32 and 0.63, respectively, ($p=0.000026$). In the same time we detected a significant reduction of ABCA1 mRNA level in leukocytes of patients with atherosclerosis when compared with controls (0.63 and 0.92, respectively, ($p=0.02$)). In the control group ABCA1 gene expression level in macrophages did not differ from the one in leukocytes. We propose that the ABCA1 mRNA level is a key factor in the development of atherosclerosis.

Poster Session

FIRST DEMONSTRATION OF THE LOCALIZATION OF HEPATIC APOB100- AND INTESTINAL APOB48-CONTAINING LIPOPROTEIN PARTICLES IN HUMAN ATHEROSCLEROTIC PLAQUES USING DUAL-STAINING IMMUNOFLUORESCENCE

Jesus Gustavo Vazquez Figueroa[1], P. Battey[1], M. Kinoshita[1], I. Marvasty[1], B. Blackman[2], A. Pryor[2], T. Matsushima[1], T. Teramoto[1], S. Rinehart[1], S. Voros[1]

[1]Cardiovascular Imaging, Piedmont Heart Institute, Atlanta, GA, [2]University of Virginia, Charlottesville, VA, USA

Introduction: We previously demonstrated the localization of both apoB100- and apoB48 in human atherosclerotic plaques by immuno-peroxidase single-staining. To further explore differential localization, we performed dual-staining using immunofluorescent technique.

Methods: We stained human small intestine (n=3) as controls and stained human carotid atherosclerotic plaques (n=8) obtained during endarterectomy. For primary antibodies we used commercial antibodies against apoB100 (ab50069) and apoB48 (Shibayagi; AKHB48E). Secondary antibodies were also commercial (Invitrogen; anti-mouse; anti-rabbit). Slides were photographed on a Nikon microscope, optimized for immunofluorescent staining.

Results: In the small intestine, we found apoB48 in the enterocytes lining the mucosa, mostly in the apical surface. ApoB100 staining was minimal or absent in the enterocytes. The tunica submucosa also had significant apoB48 staining. Importantly, the lymph had strong apoB48 staining. Smooth muscle cells in the muscularis propria were positive for apoB100 and apoB48. Dual immunofluorescent staining of the human carotid plaques revealed the presence of both apoB-100 and apoB48. ApoB48 was mostly localized in the subendothelial regions and the necrotic core. Overall, there appeared to be larger area of the plaque that was positive for apoB48, compared to apoB100.

Conclusions: Both hepatic apoB100- and intestinal apoB48-containing particles can be demonstrated in human atherosclerotic plaques and may contribute to the disease process.

Poster Session

DIFFERENTIAL LIPID/LIPOPROTEIN RESPONSE IN WOMEN VS MEN IN PRIMARY AND SECONDARY PREVENTION TREATED WITH STATINS

Giuliana Mombelli[1], N. Fabbri[1], S. Frasson[1], C. Baldo[1], V. De Vergori[1], M. Triolo[1], C.R. Sirtori[1,2]

[1]University Center for Dyslipidemias, Niguarda Hospital, [2]Department of Pharmacological Sciences, University of Milano, Milano, Italy

Background and aim: Cholesterol management in women may be less successful than in men, because of possible underutilization of lipid lowering therapy (Miller et al, Arch Int Med 160, 343, 2000), as well as possibly because of a reduced response, in particular to statins (Mehner et al Eur J Clin Pharmacol 64, 815, 2008). Practical therapy indicates that, at times, LDL-C reduction in women may be less dramatic, because of the concomitantly elevated HDL-C levels.

Methods and results: In our Clinical Center we investigated 290 women and 285 men, treated with a variety of statins (predominantly rosuvastatin in males and atorvastatin in females, at standard therapeutic doses). 43.5 % of males were in secondary prevention vs 23.4% of females; a higher percentage of females (68.6%) were hypertensive vs only 43.9% of males. At baseline, total cholesterol and LDL-C levels were similar in males and females (LDL-C 185.2±47.3 mg/dl in males vs 187.9±41.6 mg/dl in females). After statins at equivalent drugs/doses LDL-C levels went to 140.3±37.3 mg/dl in women, ie -18.7±54.9%, versus 127.8±36.1 mg/dl (-30.5±21.4%) ($p < 0.001$ between sexes). No other significant lipid/lipoprotein changes were recorded.

Conclusions: Women have a reduced cholesterolemic response to statins, not because of reduced compliance/underutilization, but most likely because the elevated HDL-C levels may impair the LDL-C lowering effect. These findings indicate that in women the unclear vascular benefit noted in some preventive trials may possibly be linked to the different baseline lipoprotein patterns between the two sexes.

Poster Session

LONGITUDINAL ASSESSMENT OF DYSLIPIDEMIA AMONG PATIENTS TREATED WITH LIPID MODIFYING THERAPY IN UK CLINICAL PRACTICE

Baishali Ambegaonkar[1], S. Eaton[2], K. Jameson[3], V. Amber[3], P. Durrington[4]

[1]Merck and Co., Inc., Whitehouse Station, NJ, [2]General Practice Research Database, Raleigh, NC, USA, [3]Merck Sharp & Dohme Ltd., Hoddesdon, [4]University of Manchester, Manchester, UK

Objective: This study evaluated the attainment of target/normal lipid levels among users of lipid modifying therapy (LMT) in a cohort of high-risk patients from U.K. general practice.

Methods: Using electronic medical records from the General Practice Research Database (GPRD), we identified 25,409 patients at high-risk of CV event, aged, 35+, who initiated LMT between April 2006-October 2007, continued treatment for 1 year, and had a complete lipid panel prior to and 1 year post LMT-initiation. A complete lipid panel consisted of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). High-risk and target/normal lipid levels were defined as per the Joint British Societies guidelines.

Results: Mean age of the sample was 65 years (~50% male). Prior to LMT-initiation, 98.3%, 92.9%, 16.6% and 47.7% of patients had elevated TC, elevated LDL-C, low HDL-C and elevated TG, respectively. HDL-C and/or TG abnormalities existed in 52.2% of patients. Nearly all patients (97.7%) initiated statin monotherapy. Post LMT-initiation, 34.7% and 65.6% of patients achieved TC and LDL-C targets, respectively. However, only an additional 17.9% attained a normalized TG level, with a small (1.3%) increase in patients with HDL-C abnormality. HDL-C and/or TG abnormalities existed among 39% of patients.

Conclusions: In this longitudinal study, patients initiating LMT largely did so with statins, and a large proportion of patients achieved LDL-C target. However, there was only a moderate reduction in TG abnormality and a slight increase in HDL-C abnormality. More than one-third of patients continued to experience HDL-C and/or TG abnormalities.

Poster Session

STRUCTURE-FUNCTION RELATIONSHIPS OF APOLIPOPROTEIN A-I MIMETIC PEPTIDES: IMPLICATIONS FOR ANTI-ATHEROGENIC ACTIVITIES OF HIGH DENSITY LIPOPROTEIN

Dmitri Sviridov[1], W. D'Souza[1], J.A. Stonik[2], A. Murphy[1], S.J. Demosky[2], A.A. Sethi[2], J. Chin-Dusting[3], A.T. Remaley[2]

[1]Lipoproteins and Atherosclerosis, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia, [2]Lipoprotein Section, National Heart, Lung and Blood Institute, NIH, Bethesda, MD, USA, [3]Vascular Pharmacology, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

Apolipoprotein A-I (apoA-I) mimetic peptides are a promising type of anti-atherosclerosis therapy, but how the structural features of these peptides relates to the multiple anti-atherogenic functions of high density lipoprotein (HDL) is poorly understood. Twenty two bi-helical apoA-I mimetic peptides were investigated *in vitro* for the capacity and specificity of cholesterol efflux, inhibition of inflammatory response of monocytes and endothelial cells and inhibition of low density lipoprotein (LDL) oxidation. It was found that mean hydrophobicity, charge, size of hydrophobic face and angle of the link between the helices are the major factors determining the efficiency and specificity of cholesterol efflux. The peptide with optimal parameters was more effective and specific towards cholesterol efflux than human apoA-I. Charge and size of hydrophobic face were also the major factors affecting anti-inflammatory properties, but optimal values for these parameters were different to those optimal for cholesterol efflux. Presence of cysteine and histidine residues was the main factor determining anti-oxidant properties. There was no significant correlation between capacities of the peptides to support individual anti-atherogenic functions and each function had its own optimal set of features. None of the peptides was equally effective in all the anti-atherogenic functions tested, suggesting that different functions of HDL may have different mechanisms and different structural requirements. The results do suggest, however, that the “Rational Design” of apoA-I mimetic peptides may substantially improve their therapeutic value and may lead to a better understanding of the anti-atherogenic functions of HDL.

Poster Session

LOVASTATIN ENHANCE PARAOXONASE ENZYME ACTIVITY AND PROLONG LOW DENSITIY LIPOPROTEIN PEROXIDATION LAG PHASE IN TYPE II DIABETIC NEPHROPATHY

Nariman Nezami[1], A. Ghorbanihaghjo[1], H. Argani[1], J. Safa[2], N. Rashtchizadeh[1]

[1]Drug Applied Research Center, [2]Department of Nephrology, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Many studies showed the role of elevated lipids and increased oxidative stress in the progression of diabetic nephropathy (DN) and cardiovascular events. We aimed to study the effect of lovastatin on low-density lipoprotein (LDL-C) susceptibility to in vitro oxidation, paraoxonase (PON) and aryle estrase (ARE) activities in patients with DN.

Methods: During present quasi clinical trial, 30 patients with type II DN were enrolled. lovastatin (20mg/day) administered for all of patients for 90 days and after that lovastatin was withdrawn for 30 days. PON and ARE activities were measured by spectrophotometeric method at 412 and 270 nm, respectively. Susceptibility of LDL-C to in vitro oxidation (lag-phase) was determined as the production of conjugated dienes induced by Cu^{2+}/5 min at 234 nm.

Results: After 90 days lovastatin intervention, Cholesterol (TC) and LDL-C levels significantly decreased and high-density lipoprotein (HDL-C) level increased significantly ($p< 0.001$, 0.010 & 0.008), despite the unchanged level of triglyceride (TG). ARE and PON activities and LDL-C lag-phase were significantly increased ($p=0.002$, 0.004, & < 0.001). After 30 days of withdrawal, TC, TG and LDL-C levels were significantly increased ($p< 0.001$, 0.010, < 0.001), but HDL-C level was not changed. Also Changes of ARE and PON activities and LDL-C lag phase were not significantly changed.

Conclusion: After 90 days of lovastatin therapy, lipid profile modified, ARE and PON activities increased and LDL-C susceptibility to peroxidation decreased. Although withdrawal of lovastatin results in worsen lipid profile, ARE and PON activities, and LDL-C susceptibility to peroxidation were not changed.

Poster Session

FAT OVERLOAD INFLUENCES LIPOGENIC FACTORS IN THE METABOLIC SYNDROME

Maribel Queipo-Ortuño[1], M.D. Mayas[2], M. Clemente-Postigo[1], R. El Bakay[2], M. Macias[3], F.J. Tinahones[4], F. Cardona[3]

[1]Hospital Clinico Universitario Virgen de la Victoria (Fundacion IMABIS), [2]Hospital Clinico Universitario Virgen de la Victoria (Fundacion IMABIS). CIBEROBN, Malaga, [3]Endocrinologia y Nutrición, Hospital Clínico Universitario Virgen de la Victoria (IMABIS), [4]Endocrinologia y Nutrición, Hospital Clínico Universitario Virgen de la Victoria (IMABIS).CIBEROBN, Málaga, Spain

Introduction: Several epidemiologic studies have related postprandial lipemia to risk of cardiovascular disease through enzymes of fatty acid (FA) metabolism as FASN, FATP or SCD1. Some transcription factors which are involved in FA metabolism are SREBF1, LXRa or RXRa. It is well known the interaction between nutrition and the human genome that determines gene expression and the metabolic response.

Objectives. Due to the fact that nutrients interact with transcription factors that regulate genes and this is not well established in vivo, the aim of this work was to evaluate influence of fat overload on the gene expression of lipogenic regulators in peripheral blood mononuclear cells (PBMC) of patients with MS.

Methods: 22 patients with criteria for MS were included in this study. All of them underwent 60g fat overload with a commercial preparation. Baseline and after fat overload we studied anthropometrical, biochemical variables and gene expression of lipogenic factors.

Results: We found the fat overload led to an increase of the expression of SREBF1, RXRa and LXRa in PBMC, and this increase was associated to the expression of FASN. In addition, we observed that triglyceride levels, correlated with FASN expression and there is a positive correlation of SREBF1 with RXRa and of LXRa with the lipoperoxides plasma concentration in the baseline or fat overload state.

Conclusions: Fat overload led to an increase of regulators of lipogenesis in PBMC in patients with MS. It is possible to use this model in order to study the response of lipid metabolism in vivo.

Poster Session

SIMULTANEOUS DETERMINATION OF TRIGLYCERIDE AND GLUCOSE TOLERANCE BY A NOVEL SEQUENTIAL TEST PROTOCOL IN 511 PATIENTS WITH CORONARY ARTERY DISEASE

Christian Werner, A. Filmer, S. Groenewold, M. Fritsch, M. Böhm, U. Laufs

Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg, Germany

Background: Epidemiologic studies suggest an association of post-prandial triglyceride (TG) levels with the risk of cardiovascular events, whereas fasting TG levels may be less predictive. Our study prospectively evaluates the role of post-prandial TG metabolism in patients with coronary artery disease (CAD).

Methods and results: Pilot experiments in 25 healthy subjects and 35 patients with CAD demonstrated that only a sequential oral triglyceride and glucose tolerance test (OTTT->OGT, 75g fat applied 3 hours prior to 75g glucose) reliably determines TG and glucose tolerance at the same time. N = 511 consecutive patients with stable CAD were subjected to the OTTT->OGT, patients with diabetes received the OTTT. Within the CAD patients, individuals without metabolic syndrome (MS) and normal glucose tolerance (n = 111) showed the lowest TG values. Patients with CAD + MS and impaired glucose tolerance / diabetes (IGT/DM) (n = 246) had the highest TG values. CAD patients with MS but no IGT/DM (n = 56) and patients with IGT/DM but no MS (n = 98) showed a significantly lower postprandial TG increase. Importantly, in all patient groups the relative post-prandial TG increase did not correlate to the absolute TG increase.

Conclusions: Metabolic syndrome and diabetes are correlated with elevated fasting and post-prandial TG levels. CAD patients show marked interindividual differences between their absolute and relative post-prandial TG response. Follow-up examinations will show whether parameters of post-prandial triglyceride metabolism are independent predictors of future cardiovascular events.

Poster Session

PARAMETERS OF THE LIPID PROFILE AND ASSOCIATION WITH THE DEVELOPMENT OF RE-OBSTRUCTION AFTER STENTIMPLANTATION IN THE SFA IN PAD-PATIENTS

Thomas Gary[1], P. Rief[1], T. Stojakovic[2], H. Froehlich[1], H. Scharnagl[2], F. Hafner[1], E. Pilger[1], M. Brodmann[1]

[1]Angiology, [2]Division of Laboratory Medicine, Medical University Graz, Graz, Austria

Background: High ApoB is described as a risk factor for the development of MACE. In the peripheral arteries elevated levels of Lpa are described as risk factor for the development of re-obstruction and re-occlusion after endovascular treatment. The aim was to evaluate the association between plasma lipid-levels and development of instent-restenosis and re-occlusion in patients with PAD and endovascular treatment with stent-implantation in a prospective setting.

Methods: 139 patients with stenting of the SFA were included in our study. Plasma lipid-levels were measured 3 months after stentimplantation. Instentrestenosis was assessed with duplex-scan of the SFA 3, 6 and 12 months after stent-implantation.

Results: 72 patients developed restenosis > 50% after stent-implantation in the SFA during 1 year, ten of these patients developed stentocclusion. In the patients developing restenosis the mean ApoB-level was 105.8 versus 94.9 in patients who did not develop re-obstruction ($p< 0.05$). Patients who did not develop re-obstruction had higher HDL-levels (mean 39.7 versus 34.7 with re-obstruction, $p < 0.05$). We devided patients developing re-obstruction in two groups: 28 patients with high-grade re-obstruction (75% to 99%) and ten patients with re-occlusion. Patients developing re-occlusion had higher levels of cholesterol (mean 234.1 versus 185.9, $p < 0.05$), plasma-Apo B (mean 135.3 versus 99.8, $p < 0.05$), LDL (mean 160.3 versus 113.6, $p < 0.05$), and LDL-Apo B (mean 115.5 versus 82.39, $p < 0.001$).

Conclusion: An imbalance in the lipid-profile could be a mayor reason for the development of re-obstruction and re-occlusion after stenting of the SFA.

Poster Session

DISCOVERY OF A NOVEL AND POTENT CHOLESTEROL ABSORPTION INHIBITOR AS PROMISING ORALLY ACTIVE LIPID LOWERING DRUG

Masanobu Kanou[1], Y. Nishiwaki[2], A. Nakano[2], Y. Yamanaka[2]

[1]Pharmaceutical Development Research Laboratories, [2]Pharmaceutical Discovery Research Laboratories, Teijin Pharma Ltd, Tokyo, Japan

Objectives: Ezetimibe, known as a cholesterol absorption inhibitor, is used for the treatment of dyslipidemia, however, its monotherapy is not enough effective to lower the lipid. We have screened many azetidinone derivatives and found a potent cholesterol absorption inhibitor TEI-M01361. We have also investigated the contrasting profile of TEI-M01361 vs. Ezetimibe.

Methods: *In vivo*: Cholesterol-lowering effect was investigated in male golden Syrian hamsters on the cholesterol-enriched diet (CE-2 diet supplemented with 0.5%cholesterol) for 7 or 28 days. From the start of feeding on the cholesterol-enriched diet, compounds are administered by gavage once daily. Four hours after last administration, whole blood is collected and serum is separated from blood. Further, livers are harvested and parts of the livers are extracted for lipid analysis. *In vitro*: We conducted inhibition assay of cholesterol absorption in human intestine Caco-2 cells and cholesterol efflux assay in human hepatoma HepG2 cells using [^{14}C]-cholesterol.

Results: TEI-M01361 showed more potent lowering effect on LDL-cholesterol and liver-cholesterol than Ezetimibe in cholesterol-fed hamsters. In human intestine Caco-2 cells, TEI-M01361 inhibited cholesterol absorption. Furthermore, TEI-M01361-glucuronide significantly enhanced cholesterol efflux than Ezetimibe-glucuronide at HepG2 cells.

Conclusions: TEI-M01361 was indicated as a highly potent cholesterol absorption inhibitor. Moreover it was intriguingly implied to promote cholesterol efflux from liver. These findings suggest that in humans, TEI-M01361 has a potential of lowering cholesterol more effectively. Therefore, TEI-M01361 is promising that it can play more effective role in the treatment of dyslipidemia.

Poster Session

ADIPONECTIN ENHANCES *DE NOVO* HEPATIC HDL SYNTHESIS THROUGH LXR ALPHA- AND COUP-TFII-DEPENDENT PATHWAYS

Kazumi Tsubakio-Yamamoto[1], T. Ohama[1], D. Masuda[1], J. C. Sandoval[1], M. Inagaki[1], K. Nakatani[1], M. Yuasa-Kawase[1], R. Kawase[1], H. Nakaoka[1], Y. Nakagawa-Toyama[2], M. Nishida[2], M. Ishigami[3], S. Kihara[4], I. Shimomura[4], I. Komuro[1], S. Yamashita[1]

[1]Department of Cardiovascular Medicine, [2]Health Care Center, [3]Department of Biomedical Informatics, Division of Health Sciences, [4]Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Suita-shi, Japan

Background: HDL and apolipoprotein A-I (ApoA-I) take up cholesterol from peripheral tissues and transport it back to the liver, a system called “reverse cholesterol transport (RCT)”. Both ApoA-I and ATP-binding cassette transporter A1 (ABCA1) are the rate-limiting factors that generate HDL in the liver. We found a positive correlation between plasma HDL-C and adiponectin (APN) concentrations in humans, and plasma APN levels were decreased in patients with coronary artery disease (CAD) and metabolic syndrome. We have shown that APN accelerates RCT by increasing the hepatic expression of ApoA-I and ABCA1. In the current study, we further evaluated the molecular mechanism of APN-induced enhancement of ApoA-I and ABCA1expression by testing whether COUP-TFII, one of the nuclear receptors, and LXR-alpha are involved in this mechanism.

Methods: A human hepatoma cell line, HepG2 cells, were incubated for 20 h in the culture medium containing recombinant human APN after the incubation for 12 h with siRNA or control RNA for COUP-TFII or LXR-alpha and the mRNA levels of ApoA-I and ABCA1 were measured by real time PCR.

Results: APN up-regulated the mRNA and protein levels of ApoA-I and ABCA1 in HepG2 cells. Furthermore, the expressions of COUP-T**F**II and LXR-alpha were also significantly increased by APN, while these expression levels were significantly lower in APN-knockout compared with wild-type mice. In HepG2 cells, the reduction of COUP-TFII by siRNA reduced the APN-induced enhancement of ApoA-I but not ABCA1.

Conclusions: APN might protect against atherosclerosis by enhancing *de novo* hepatic HDL synthesis through both COUP-TFII- and LXR-alpha-dependent pathways.

Poster Session

COMPARISON OF METHODS FOR MEASURING LDL-CHOLESTEROL IN THE FASTING AND NONFASTING STATE IN PATIENTS WITH TYPE-2 DIABETES MELLITUS

Søren Lund[1], M. Petersen[2], M. Frandsen[1], U. Smidt[1], H.-H. Parving[3], A. Vaag[1], T. Jensen[1]

[1]Steno Diabetes Center, [2]Faculty of Life Sciences, University of Copenhagen, Gentofte, [3]Rigshospitalet, Copenhagen, Denmark

Background and aims: Type-2 diabetes (T2DM) patients have an excess risk of cardiovascular disease (CVD). LDL-cholesterol (LDL-C) is a modifiable CVD risk factor. The use of fasting or postprandial samples and the best method for measuring LDL-C are under debate. We studied the agreement between fasting and postprandial LDL-C in T2DM-patients and compared LDL-C methods.

Methods: Seventy-four T2DM-patients were served a test-meal. Blood was sampled at fasting and 1.5, 3.0, 4.5 and 6.0 hours postprandially. LDL-C was measured with modified beta-quantification (MBQ), Friedewald equation (FE) and a direct assay (DA). Agreement with 95% limits of agreement (LOA) at ±0.20 mmol/l was evaluated.

Results: LDL-C levels at all postprandial times disagreed with those at fasting for all methods. Maximum mean [95% LOA] differences were: −0.16 mmol/l [−0.52; 0.20]; −0.37 mmol/l [−0.89; 0.16] and −0.26 mmol/l [−0.66; 0.14] with the MBQ, FE and DA, respectively. In postprandial samples, FE misclassified 38% of patients (64% of statin users) into lower ATP-III risk categories. Female gender and a postprandial triglyceride level >2.08 mmol/l each indicated greater disagreement between fasting and postprandial LDL-C (interactions: p≤0.038). The MBQ disagreed with FE and DA, especially in postprandial samples (maximum mean [95% LOA] differences versus MBQ: FE: −0.35 mmol/l [−0.92; 0.22]; DA: −0.41 mmol/l [−0.83; 0.00]).

Conclusions: There is a potentially clinically relevant disagreement (approaching 1.0 mmol/l) *within* (fasting versus postprandial) and *between* LDL-C methods in T2DM-patients. These findings do not support the routine use of nonfasting samples and cast doubt on the diagnostic value of LDL-C methods.

Poster Session

EFFECTS OF FENOFIBRIC ACID IN COMBINATION WITH STATIN THERAPY ON LOW-DENSITY LIPOPROTEIN PARTICLE SIZE IN PATIENTS WITH MIXED DYSLIPIDEMIA

Darryl J Sleep[1], I.S. Mandair[2], C.M. Setze[1], M.T. Kelly[1]

[1]Abbott, Abbott Park, IL, USA, [2]Solvay Pharmaceuticals, Allschwil, Switzerland

Background: Larger LDL particles (pattern A) are associated with less cardiovascular risk than smaller, denser particles (pattern B).

Purpose: To evaluate the effect of fenofibric acid (FA) + statin combination therapy on LDL particle size.

Methods: In three double-blind phase 3 studies, 2453 patients with LDL-C ≥130 mg/dL, TG ≥150 mg/dL, and HDL-C < 40 mg/dL (< 50 for females) received FA (135 mg); low-dose statin (LDS; simvastatin or atorvastatin 20 mg or rosuvastatin 10 mg); moderate-dose statin (MDS: simvastatin or atorvastatin 40 mg or rosuvastatin 20 mg) or the combination of FA + LDS or MDS for 12 weeks. Change in LDL particle size (NMR) was evaluated in a subset of 775 patients. Patients were categorized into pattern A (20.6-23.0 nm) or pattern B (18.0-20.5 nm) based on mean LDL particle diameter at baseline and on therapy.

Results: Combination therapy increased the proportion of patients with LDL particle pattern A from 11.0% to 49.3% (FA + LDS) and 11.1% to 44.4% (FA + MDS) compared to statin monotherapy (9.8% to 17.0% [LDS] and 13.6% to 18.3% [MDS]).

Means, LDL particle size	FA Monotherapy (n = 154)	LDS Monotherapy (n = 153)	FA + LDS (n = 146)	MDS Monotherapy (n = 169)	FA + MDS (n = 153)
Baseline (nm)	20.0	19.9	19.8	19.9	19.9
Final Visit (nm)	20.5	20.1	20.5	20.1	20.5
% change	2.9%	0.9%	3.2%; P<0.001 vs LDS	1.0%	2.7%; P<0.001 vs MDS

[% Change in LDL Particle Size]

Conclusions: Combination therapy with fenofibric acid and a statin resulted in the greatest increases in LDL particle size and the highest percent of patients with a favourable LDL pattern.

Poster Session

SUSTAINED RELEASE NICOTINIC ACID VERSUS INOSITOL HEXANICOTINATE FOR THE TREATMENT OF DYSLIPIDEMIA

Joseph Keenan

Dept. of Family Medicine, University of Minnesota, Minneapolis, MN, USA

A comparison of the efficacy, safety of two forms of nicotinic acid: wax-matrix, sustained release, nicotinic acid (NA) and Inositol Hexanicotinate (IHN), or “no flush” niacin. This was a 6 week, 3 parallel arm (N = 120, 40 subjects per arm), clinical trial using 1500mg/d (500mg three times a day) of NA, IHN, and placebo. Inclusion criteria: baseline LDL (130- 190mg%); HDL < 60mg% and TG < 400mg%. Subjects had fasting lipids, chemistries, and side effect questionnaire at baseline, end of week 3 and end of the study. Lipid results for NA (TC = -11%, ($p < .001$); LDL = -18%, ($p < .001$), and HDL = + 12% ($p < .001$), and TG (-9%, $p = .074$). Lipid results for IHN (TC = -1%; LDL = -1%; HDL = +1%; TG = +2%) and placebo (TC = 0%; LDL = 0%; HDL = +2% ; TG = -1%) were not significant. All treatments were well tolerated; only one drop out from the NA due to side effects. Compliance with treatment was good, over 92% of the treatment doses for all groups and dietary compliance (3 day food records) was good. The NA group did show a slight increase in blood glucose, homocystiene, and liver enzyme levels, but levels stayed within the range of normal. In conclusion, wax matrix sustained-release NA is a beneficial and well tolerated intervention for dyslipidemia and IHN was well tolerated, but appears to be no better than placebo.

Poster Session

DISTINCT PLASMA FATTY ACIDS ASSOCIATE WITH COMPONENTS OF THE METABOLIC SYNDROME IN HEALTHY OFFSPRING OF TYPE 2 DIABETES PATIENTS

Ludger Scheja, M. Vossen, K. Toedter, U. Beisiegel

University Hospital Hamburg-Eppendorf, Hamburg, Germany

Objective: The identification of fatty acids as metabolic syndrome biomarkers in healthy offspring of type 2 diabetes patients.

Methods: Type 2 diabetes offspring (n=91) without clinical disease and with different degrees of insulin sensitivity, as assessed by HOMA-IR, were recruited. Plasma samples were collected in the fasted state and 2 hours after an oral glucose load. Subsequently, metabolic profiling including fatty acids, lipoproteins, hormones, adipokines and inflammation markers was performed. Data were explored statistically by correlation analysis.

Results: We observed numerous correlations of fatty acids with insulin resistance and metabolic syndrome parameters. Interestingly, some fatty acids correlated significantly with fasting glucose. Among those, dihomo-gamma-linolenic acid (DGLA) showed a negative correlation with fasting glucose, however, a positive correlation with plasma insulin. This correlation pattern may reflect stimulation of DGLA synthesis by insulin and suppression of hepatic glucose production by DGLA, respectively. Docosahexaenoic acid (DHA) showed a specific, positive correlation with glucose 2h into the glucose tolerance test, suggesting a link of DHA to peripheral insulin resistance and glucose disposal.

Conclusions: Our data reveal novel associations of fatty acid with metabolic syndrome parameters, especially such with plasma glucose, and they suggest novel mechanisms connecting fatty acid and glucose metabolism.

Poster Session

RELATIVE RESPONSIVENESS TO LDL-LOWERING AGENTS THAT BLOCK CHOLESTEROL ABSORPTION (EZETIMIBE) AND INHIBIT CHOLESTEROL SYNTHESIS (SIMVASTATIN)

S.G. Lakoski[1], G.L. Vega[1], S.M. Grundy[1], M. Chandalia[2], C. Lam[3], **Robert S Lowe**[3], M.E. Stepanavage[3], T.A. Musliner[3], J.C. Cohen[1], H.H. Hobbs[1]

[1]Donald W. Reynolds Cardiovascular Clinical Research Center, University of Texas Southwestern Medical Center, Dallas, [2]University of Texas Medical Branch, Galveston, TX, [3]Merck & Co., Inc., North Wales, PA, USA

Introduction: Levels and duration of exposure to circulating LDL-cholesterol (C) are major contributors to CHD. Therefore, optimal prevention will require long-term LDL-C reduction, making it important to select the most effective agent for each individual. Here we tested the hypothesis that individuals with high rates of cholesterol absorption will respond better to ezetimibe and less well to simvastatin; conversely, 'low absorbers' with high rates of cholesterol synthesis will respond better to simvastatin than ezetimibe.

Methods: Randomized, double-blind, placebo-controlled, cross-over trial with 215 men receiving ezetimibe (10 mg), simvastatin (10 mg), or both drugs daily for 6 weeks. Baseline and post-treatment plasma levels of surrogate markers for cholesterol absorption (campesterol) and synthesis (lathosterol), and PCSK9 (involved in LDL receptor regulation) were measured.

Results: Baseline LDL-C was 146±20mg/dL. Ezetimibe, simvastatin and combination therapy reduced LDL-C by 19%, 25% and 41%, respectively. Correlation coefficients between percent LDL-C reduction and baseline levels of non-cholesterol sterols were uniformly low (all ≤0.18). PCSK9 levels did not correlate significantly with LDL-C reduction in any group. Although individual responses were variable, percent change in LDL-C levels on ezetimibe was directly related to the response to simvastatin ($r=0.46$, $p< 0.001$).

Conclusion: Baseline plasma levels of PCSK9 and noncholesterol sterols were both poor predictors of responses to simvastatin, ezetimibe, or both drugs. Unexpectedly, the percent reductions in LDL-C levels with simvastatin and ezetimibe were highly and directly correlated, suggesting that shared factors that are independent of the major sites of action of these drugs contribute importantly to the therapeutic response.

Poster Session

EFFECT OF PROBUCOL ON AN ANTIOXIDANT PROPERTY OF HDL IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

Miwako Inagaki[1], Y. Nakagawa-Toyama[1,2], R. Kawase[1], H. Nakaoka[1], K. Nakatani[1], M. Yuasa-Kawase[1], K. Tsubakio-Yamamoto[1], J. C Sandoval[1], D. Masuda[1], T. Ohama[1], M. Nishida[1,2], M. Ishigami[3], S. Yamashita[1]

[1]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, [2]Health Care Center, Osaka University, Toyonaka, [3]Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, Suita, Japan

High density lipoprotein (HDL) has multi-antiatherogenic effects such as antioxidation, antiinflammation and anticoagulation, in addition to being a key mediator of reverse cholesterol transport. Probucol, known as a lipid lowering drug, is also a potent antioxidant, which raised a question whether probucol could be involved in antioxidant capability of HDL. To address this question, we investigated effect of probucol on an antioxidant feature of HDL in patients with heterozygous familial hypercholesterolemia (FH) which is an inherited disorder characterized by rapid atherosclerosis progression. Probucol-treated FH patients (P+, n=19) showed 47% reduction of plasma HDL cholesterol (HDL-C) level compared to probucol-non-treated FH patients (P-, n=15), whereas there was no significant difference in plasma low density lipoprotein-C (LDL-C) level. HPLC analysis revealed that peak HDL-lipid levels in P+ were shifted to the right against those in P-, which proved HDL particle size got smaller in P+. AAPH induced LDL oxidation was employed to evaluate the capacity of HDL to protect LDL from oxidation. HDL derived from P+ significantly prolonged lag phase duration by 112%, decreased maximum oxidation rate by 23%, and lowered maximum concentration of conjugated dienes formation by 16%. In conclusion, smaller size HDL induced by probucol exhibited more antioxidative activity than that without probucol treatment in patients with heterozygous FH, indicating that HDL antioxidative property could be incremented by probucol.

Poster Session

ENVIRONMENTAL RISK FACTORS LEADING TO HYPERTRIGLYCERIDEMIA ON HETEROZYGOUS LIPOPROTEIN LIPASE (LPL) DEFICIENCY IDENTIFIED IN THE GENERAL POPULATION OF JAPANESE

Yasuyuki Ikeda, A. Takagi, N. Iwai, Y. Kokubo, H. Tomoike

National Cardiovascular Center, Suita, Japan

Objectives: Our aim is to elucidate effect on the expression of hypertriglyceridemia (HTG) and association with ischemic heart disease (IHD) of heterozygous LPL deficiency identified in a general Japanese population (Suita Study).

Methods: The 3650 Japanese subjects (1705 M and 1945 F), who were selected randomly from the municipal population registry in Suita in Osaka in Japan, were studied. Laboratory and biochemical tests were performed using blood samples obtained after overnight fast. Genotype of the 24 LPL mutations, identified in the Japanese, was examined with TaqMan Method. Statistical studies on the identified 11 LPL heterozygotes and LPL normal 3639 subjects were examined by univariate and multivariate logistic analyses.

Results: In the LPL normal subjects, three environmental risk factors such as abdominal obesity, a high alcohol drinking habit, and impaired fasting glucose were independently associated with the expression of HTG, and additive effects of the three factors on the expression of HTG were observed in a stepwise fashion for both male and female. HTG and hypertension were independent risk factors for IHD and subjects with at least both risk factors had a 2.44-fold OR (95% CI, 1.39-4.29) for IHD. In the LPL heterozygotes (n=11), even a few burdens of environmental risk factors predisposed them to mild or moderate HTG in 4.99-fold OR (95% CI, 1.52-16.41), and in fact, one proband with old IHD was detected from the heterozygotes (n=6) with HTG.

Conclusions: Heterozygous LPL deficiency is prone to increase the expression of HTG and may thereby predispose its carriers to IHD.

Poster Session

TRANS ELAIDIC BUT NOT VACCENIC ACID IMPAIRS ABCA1-DEPENDENT CHOLESTEROL EFFLUX FROM J774 MACROPHAGES

Natalie Fournier[1,2], N. Attia[1], D. Rousseau[1], B. Vedie[2], F. Destaillats[3], A. Grynberg[1], J.-L. Paul[1,2]

[1]UMR INRA 1154, Faculté des Sciences Pharmaceutiques, Université Paris-Sud 11, Châtenay-Malabry, [2]Laboratoire de Biochimie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France, [3]Centre de Recherche, Nestlé, Vevey, Switzerland

The mechanisms explaining the atherogenicity of trans fatty acids (TFA) are still unclear and their deleterious effects may be modulated by their origin. We investigated the impact of TFA incorporation on ABCA1-mediated cholesterol efflux from cAMP pretreated macrophages. J774 mouse cells were radiolabeled with [3H]-cholesterol and exposed during 30h to media enriched or not (standard medium) with either Elaidic Acid (trans 18:1 delta 9; EA) usually provided by hydrogenated vegetable oils or Vaccenic Acid (trans 18:1 delta 11; VA) usually provided by natural dairy products bound to bovine serum albumin (90 µmol/L, BSA molar ratio of 1.8). Compared with standard medium, EA reduced cholesterol efflux to free apolipoprotein A1 (10 µg/mL, 4h) by 23% (n=5 experiments) while VA induced a slight and not significant cholesterol efflux reduction of 9%. The expression of ABCA1 (mRNA and protein) was not altered by TFA supplementation. The Gas Liquid Chromatography analysis of membrane phospholipids composition revealed that EA and VA represent 19% and 9% of total FA, respectively and that their incorporation induced a decrease in the ratio ((monounsatured FA+ polyunsaturated FA) / (saturated FA+ trans FA)) (MUFA+PUFA) / (SFA+trans). Moreover, in dose-response experiments, a high positive correlation was observed between (MUFA+PUFA) / (SFA+trans) index and cholesterol efflux (r=0.84, $P< 0.01$). Our results suggest that Elaidic Acid considered as highly atherogenic contributes to atherogenesis by impairing the ABCA1 cholesterol efflux pathway in macrophages, likely by increasing the membrane rigidity which could reduce the transporter functionality.

Poster Session

THE DIAGNOSTIC ALGORITHM FOR APOB DYSLIPOPROTEINEMIAS APPLIED TO THE DUTCH POPULATION

Suzanne Holewijn[1], A. Sniderman[2], M. den Heijer[3,4], D. Swinkels[5], A. Stalenhoef[1], J. de Graaf[1]

[1]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, [2]Mike Rosenbloom Laboratory for Cardiovascular Research, McGill University Health Centre, Montreal, QC, Canada, [3]Department of Endocrinology, [4]Department of Epidemiology and Biostatistics, [5]Department of Laboratory Medicine, Laboratory of Clinical Chemistry, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Background: Recently, a new diagnostic algorithm using apolipoprotein B(apoB) in combination with triglycerides(TG) and total cholesterol(TC) was published to diagnose apoB dyslipoproteinemias, as described by HyperapoB-hyperTG(high VLDL+LDL), HyperapoB-normoTG(high LDL) and HyperTG-normoapoB(high chylomicrons or remnants or VLDL(Nat.Clin.Pract.Endocrinol.Metab.2008;4:608-618).

Aim: To determine the prevalence of the apoB dyslipoproteinemias in the general population and to characterize their overall cardiovascular (CV) risk profiles, including arterial structure and function, as measured with a panel of non-invasive parameters.

Methods: Clinical, biochemical and non-invasive measurements of atherosclerosis (NIMA) were determined in 1517 individuals from the general population, aged 50-70 years.

Results: The prevalence of hyperapoB-hyperTG was 15.1% - HyperapoB-normoTG 4.9% - normoapoB-hyperTG 18.1%(due to high VLDL) and 2.3%(high chylomicrons and VLDL). All apoB dyslipoproteinemias showed a comprised clinical and biochemical CV risk profile. Of all apoB dyslipoproteinemias, subjects with HyperapoB-HyperTG showed the most deteriorated NIMA reflecting atheroma, as indicated by decreased ABI at rest(-3.5%) and after exercise(-9.8%), increased IMT(+5.5%), and increased prevalence of plaques(+39.1%) compared to the normolipidemic control group. Subjects with NormoapoB-HyperTG due to increased VLDL showed the most deteriorated stiffness parameters reflected by increased pulse wave velocity +7.6%; augmentation index +1.7%; central systolic +4.4% and central diastolic pressure +3.7% (and intermediate deteriorated atheroma parameters).

Conclusions: The overall prevalence of apoB dyslipoproteinemias was ~ 40% in the general population. All apoB dyslipoproteinemias were characterized by a worse CV risk profile. However, the apoB dyslipoproteinemias differed in the extent of abnormality in the different NIMA compared to the reference group, suggesting differences in the main determinants of different NIMA.

Poster Session

CIRCADIAN BLOOD PRESSURE VARIABILITY, AMBULATORY ARTERIAL STIFFNESS INDEX AND ERYTHROCYTE ELECTRON TRANSFER IN SIBLINGS OF TYPE 1 DIABETES FAMILIES

Elena Matteucci, C. Consani, M.C. Masoni, O. Giampietro

Internal Medicine, University of Pisa, Pisa, Italy

Background and aims: Normotensive non-diabetic relatives of type 1 diabetics (T1D) have an abnormal blood pressure response to exercise testing that is associated with indices of metabolic syndrome and oxidative damage. We compared the pattern of 24-h ambulatory blood pressure monitoring (ABPM), ambulatory arterial stiffness index (AASI), indices of cardiovascular autonomic function, clinical and biochemical parameters, and oxidative biomarker between T1D patients, their siblings and controls.

Methods: ABPM was undertaken in 25 controls, 20 T1D and 20 siblings. In addition to laboratory examination (including homeostasis model assessment of insulin sensitivity) and clinical testing of autonomic function, we measured the rate of oxidant-induced erythrocyte electron transfer to extracellular ferricyanide (RBC vfcy).

Results: Systolic blood pressure (SBP) midline-estimating statistic of rhythm and pulse pressure were higher in T1D and correlated positively with diabetes duration and RBC vfcy; autonomic dysfunction was associated with diastolic BP ecphasia and increased AASI. Siblings had higher BMI, lower insulin sensitivity, larger SBP amplitude, and higher AASI than controls. Daytime SBP was positively independently associated with BMI and RBC vfcy. Among non-diabetic people, there was a significant correlation between AASI and fasting plasma glucose.

Conclusions: Siblings of T1D exhibited a cluster of metabolic abnormalities associated with consensual perturbations in vascular variability (abnormal diurnal profiles of SBP and abnormal dynamic relationship between SBP and DBP over 24 h as described by AASI). Moreover, our findings support, in a clinical setting, the proposed role of transplasma membrane electron transport systems in vascular pathobiology.

Poster Session

IMPACT OF RENAL INSUFFICIENCY ON DYSLIPIDEMIA IN A TRANSGENIC MOUSE MODEL

Michèle Chabert[1], X. Rousset[2], M. Lacasa[2], F. Châtelet[3], A. Kalopissis[2]

[1]Centre de Recherche des Cordeliers, UMRS 872 and Ecole Pratique des Hautes Etudes, [2]Centre de Recherche des Cordeliers, UMRS 872, [3]CHU Saint-Antoine, INSERM U538, Paris, France

Kidney insufficiency confers an increased risk of cardiovascular disease (CVD), probably linked to dyslipoproteinemia. To study the impact of renal insufficiency on dyslipidemia, we generated a transgenic mouse model of primary amyloidosis leading to glomerulosclerosis and renal insufficiency.

Transgenic mice express human apo A-II mutated in the stop codon and longer than normal apo A-II (10kDa versus 8.5kDa). Transgenic lines K and F expressing mut-apoA-II at 45 and 70 mg/dl, respectively and develop glomerular amyloidosis as a function of transgene expression level and age. Conversely, line Y with very low mut-apoA-II expression level (5-10 mg/dl) is devoid of amyloidosis. Typical amyloid depots staining with congo red are present in kidney glomeruli and adjoining capillaries, but also in liver, heart and spleen. Amyloid depots appear after age 3 months and increase with age, while apical reabsorption of proteins is progressively impaired.

Lipoproteins were analyzed by FPLC and ultracentrifugation at ages 4, 5, 6, and 7 months. Y mice displayed a normal lipoprotein profile. VLDL and remnants progressively accumulated in plasma of K and F mice, in relation with age. HDL decreased as a function of age, particularly in the high-expressing F mice. Interestingly, the amount of mut-apo A-II in HDL as well as in plasma decreased with age, again more drastically in F mice.

In conclusion, this murine model will allow to study development of hypertriglyceridemia and decrease in HDL during renal insufficiency and to assess potential impact on aortic lesion formation.

Poster Session

LYSOSOMAL DYSFUNCTION IN ATHEROSCLEROTIC HEART DISEASE

M. Greeve, G. MacIntosh, **Peter Meikle**

Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

Objectives: In the early stages of atherosclerosis (AS) cholesterol esters accumulate as lipid droplets within the cytoplasm of macrophages resulting in foam cell formation and contributing to the formation of fatty streaks. In later stages (complex fibrolipid plaques), foam cell formation also involves the accumulation of free cholesterol and cholesterol esters within lysosomes. Associated with these later stage foam cells is an increase in cellular ceramide which is thought to contribute to the inflammatory process, and ultimately to macrophage apoptosis, leading to the formation of the necrotic core and plaque instability. The cellular mechanism(s) leading to elevated ceramide have not been defined.

Methods: To investigate the role of lysosomes in the process of foam cell formation THP-1 macrophages were treated with modified LDL and changes to lipid pools measured by mass spectrometry.

Results: Acetylated LDL resulted in the accumulation of primarily cholesterol esters. In contrast, oxidized LDL caused an accumulation of primarily free cholesterol, supporting a lysosomal location. In addition, we observed a marked elevation of intracellular ceramide and a decrease in lysosomal sphingosine. Further changes were observed in the amount and fatty acid composition of the intralysosomal lipid bis(monoacylglycero)phosphate. *De novo* ceramide synthesis rate was unchanged and inhibition of reactive oxygen species production did not prevent the accumulation of ceramide, while sphingomyelinase inhibition substantially reduced ceramide accumulation.

Conclusions: These results suggest that ceramide accumulation in macrophage foam cells results primarily from a lysosomal dysfunction resulting in the inability to catabolise ceramide.

Poster Session

GLUT 4 AND THE ATGL AND HSL LIPASES ARE DOWN-REGULATED IN SUBCUTANEOUS ADIPOSE TISSUE OF PATIENTS WITH FCHL

Josep Ribalta[1], J. Salazar[1], J.C. Vallvé[1], M. Gonzàlez[1], A. Horra[1], R. Rosales[1], N. Plana[2], R. Ferré[2], L. Masana[2]

[1]Universitat Rovira i Virgili, CIBERDEM, [2]Hospital Universitari Sant Joan Reus, CIBERDEM, Reus, Spain

Introduction: The main metabolic defect of FCHL is hepatic overproduction of very low density lipoproteins (VLDL) as a result of impaired free fatty acid (FFA) trapping by the adipose tissue. The responsible molecular defects of the adipose tissue are not known and their identification would help identifying a major metabolic defect in FCHL.

Aim: We aimed at studying gene expression in the adipose tissue of FCHL patients of 24 candidate genes for impaired adipocyte function .

Patients and methods: We obtained subcutaneous adipose tissue samples from 5 FCHL patients and 5 normolipidemic controls. Real time RT-PCR was used to measure gene expression of the following genes : ATGL, PCK1, CNR1, PPARγ, LIPA, AQP7, APOC1, GPD1, PPARα, HSL, GLUT4, SCARB, LEPTIN, CRABP1, CRABP2, ALOX5AP, ADIPONECTIN, RETN, RXRg, SREBF1, SREBF2, TNFα, USF1, VISFATIN.

Results: Eight genes were significantly differentially expressed in all 5 patients compared to controls. The insulin regulated glucose transporter GLUT4 and the lipases ATGL and HSL were significantly down-regulated (mean decrease of 63%, 42% and 59% respectively) in all patients as well as GPD 1 (mean decrease of 52%). Conversely, ApoC1 which has been associated to increased cardiovascular risk was significantly up-regulated in all patients (mean increase of 234).

Conclusion: Gene expression analyses in subcutaneous adipose tissue of FCHL patients suggest decreased activity of glucose transport (GLUT4) and triglyceride hidrolysis (ATGL, HSL). The increased expression of apoC1 in all of them may help explain the increased CAD risk associated to this condition.

Poster Session

EFFICACY AND SAFETY OF A FIXED COMBINATION OF FENOFIBRATE AND METFORMIN IN CO-ADMINISTRATION WITH STATIN THERAPY. THE FAME METFO STUDY

P. Valensi[1], **Isabelle Gottlieb**[2], S. Le-Mouhaer[3], M. Conte[4]

[1]Department of Endocrinology-Diabetology-Nutrition, Jean Verdier Hospital, AP-HP, Paris Nord University, Bondy, [2]Clinical Development - Cardiometabolic, [3]Global Statistics, [4]Clinical Operations, Laboratoires Fournier SA / Solvay Pharmaceuticals, Suresnes, France

Reduction of cardiovascular risk related to diabetes requires management of risk factors including optimization of lipids control. The FAME Metfo study was an international, double-blind, phase 3, randomized study which included 482 type 2 diabetes patients treated with metformin (HbA1c ≤ 8.5%), with dyslipidemia not appropriately controlled with statin, characterized by triglycerides ≥ 1.69 mmol/L and LDL-C ≤ 3.35 mmol/L. The study compared in 2 parallel arms, during 6 months, a fixed combination of fenofibrate and metformin versus metformin. The statin therapy prior to entry in the study was kept unmodified as well as the metformin dose. Baseline characteristics were similar in both groups. Median for triglycerides was 2.44 mmol/L, and 6.70% for HbA1c. LDL-C and HDL-C means were 2.40 and 1.11 mmol/L, respectively. At study completion, there was significant difference between the 2 groups for triglycerides (LS-mean) of -28.7% and of +4.1% on HDL-C, in favor of the fixed combination, thus meeting study objective; the difference was also statistically significant for various lipid fractions, with no difference for LDL-C, which remained stable. No difference between the 2 groups was observed for HbA1c (median), fasting glucose and fructosamine. Premature withdrawals due to adverse events were similar in frequency (< 5%) and nature. No creatine kinase increase and no unexpected changes in laboratory parameters were observed. The fixed combination of fenofibrate and metformin in co-administration with statin therapy demonstrated its efficacy on lipid control without altering glucose control and without specific safety concerns when compared to metformin in co-administration with statin.

Poster Session

HDL-C RELATES TO MARKERS OF ABSORPTION BUT NOT OF SYNTHESIS OF CHOLESTEROL: STUDY IN SUBJECTS WITH LOW *VS.* HIGH HDL-C

Valéria Sutti Nunes[1], C.C. Leança[1], N.B. Pnazoldo[2], D.F. Correa[2], E.R. Nakandakare[1], E.C. de Faria[2], E.C.R. Quintão[1]

[1]Lipids Lab (LIM10), Endocrinology and Metabolism Division of Hospital das Clinicas, Faculty of Medical Sciences of the University of Sao Paulo, São Paulo, [2]Department of Clinical Pathology, School of Medical Sciences, State University of Campinas-UNICAMP, Campinas, Brazil

Introduction: HDL-C plays antiatherogenic functions that include its role in the reverse cholesterol transport but to what extent HDL-C interferes with the whole body cholesterol economy is unknown.

Objective: Measure body cholesterol synthesis (desmosterol and lathosterol) and intestinal cholesterol absorption markers (campesterol and β-sitosterol) in subjects that differ solely according to their plasma HDL-C concentration.

Methods: Healthy participants (both genders) were recruited for presenting low HDL-C (< 40mg/dL, n=38, 27 male and 11 female) and high HDL-C (>60 mg/dL, n=62, 12 male and 50 female), BMI < 30kg/m^2, after excluding secondary causes that might interfere with their plasma lipid concentrations such as smoking, heavy drinking and diabetes. Blood samples were drawn after a 12h fasting period for the measurement of sterols by the combined GLC/MS analysis utilizing 5α-cholestane as an internal standard.

Results: Plasma concentrations of HDL-C (mg/dL) and sterols (µg/mL) shown below are expressed as mean±SD and comparisons between both groups done by the unpaired Student's t test:

	Low	High
HDL-C(mg/dL)	32±7	84±16*
DESMOSTEROL(µg/mL)	0.497±0.315	0.493±0.294
LATHOSTEROL(µg/mL)	1.549±0.757	1.299±0.749
CAMPESTEROL(µg/mL)	1.454±0.649	2.679±1.300*
β-Sitosterol(µg/mL)	1.044±0.396	1.681±0.714*

[table]
*Low vs High, p< 0.0001.

Conclusion: Current data show that plasma HDL-C concentration is related to markers of intestinal absorption but not of synthesis of cholesterol.

Funding: FAPESP 07/53857-5 and 06/60585-9.

Poster Session

CHOLESTEROL- AND FATTY-ACID-PROFILES IN SUBJECTS WITH IRON OVERLOAD AND IRON DEFICIENCY ANEMIA

Nina Sauer, K. Tödter, U. Beisiegel, P. Nielsen

Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, University Medical Center Hamburg, Hamburg, Germany

Objective: Iron is an important structural or functional component of various biochemical pathways. The influence of iron on the lipid metabolism is not completely understood and often leads to controversial discussions considering for example excess iron as a cardiac risk factor (Salonen et al. 1992, Sullivan 1989). However, also severe iron deficiency could influence the formation or degradation of lipids.

Methods: Frozen serum samples from patients with iron overload (hered. haemochromatosis type 1, n=110; iron deficiency anaemia, n=43; and from a control group with normal iron status, n=79) were analyzed for serum lipoproteins (VLDL, LDL, and HDL) by fast protein liquid chromatography (FPLC) Subsequently cholesterol and triglyceride contents of the separated lipoproteins were quantified by the routine optical tests. In a subset of total serum samples and FPLC fractions, 22 different long chain fatty acids with different degrees of saturation were quantitated by gas chromatography.

Results: Both iron groups showed a clearly different FPLC-profile with lower cholesterol values in lipoproteins compared to the controls, but were quite similar to each other. The same similarity was found among fatty acid (FA) profiles in total serum samples. Saturated FAs were lower, MUFAs were similar, and PUFAs (w3 and w6) were significantly higher in iron patients than in controls.

Conclusion: This pilot study indicates that excess and deficiency of iron both can affect lipid profiles in humans plasma to almost similar degree. Further studies have to clarify which detailed mechanisms are involved in the very frequent disorders of iron metabolism.

Poster Session

LIPID TRANSFER FROM AN ARTIFICIAL LIPIDIC NANOEMULSION TO HIGH DENSITY LIPOPROTEIN IN RESISTANCE EXERCISE

Jeferson Luis Da Silva, R.C. Maranhão, V.M. Silva, C.G.C.M. Vinagre

Laboratory of Metabolism of Lipids, Heart Institute (InCor), Medical School Hospital, University of São Paulo, São Paulo, Brazil

Introduction: Lipid transfer from other lipoproteins to HDL is an important step in HDL intravascular metabolism and is involved in the functions of the lipoprotein in reverse cholesterol transfer and cholesterol esterification. The objective of this study was to evaluate whether resistance exercise training is capable of changing lipid transfers to HDL. As HDL has also anti-oxidant effects on LDL, LDL oxidation (LDL-ox) was also evaluated.

Methods: Plasma samples of 8 resistance exercise trained and 8 sedentary individuals were incubated with a lipidic nanoemulsion labeled with ^{3}H-triglycerides (^{3}H-TG), ^{14}C- cholesterol (^{14}C-C), ^{14}C-phosfolipid (^{14}C-PL) and ^{3}H-cholesteryl ester (^{3}H-CE). After chemical precipitation of apoB lipoproteins and nanoemulsion, HDL containing supernatant was counted for radioactivity.

Results: Lipid transfer (in % of total radioactivity) to HDL for the Sedentary and Resistance Exercise Group was the same for ^{14}C-Phospholipids (27.39 ± 2,21 and 26.29 ± 1.97), ^{14}C-Cholesterol ester (5.23 ± 2,44 and 7.18 ± 1.19) and ^{3}H -Free Cholesterol (9.22 ± 2,47 and 7.32 ± 1.64). Significant difference was found for ^{3}H-Triglycerides between the two groups: 6.85 ± 0,94 for the sedentary group and 4.60 ± 1.30 for the resistance exercise group.

Conclusion: Triglyceride transfer to HDL was lower in trained individuals where LDL-ox was also diminished. This result suggests that resistance exercise could have a protective role against atherosclerosis, since excess triglyceride transfer to HDL may hamper cholesterol reverse transport and by diminishing LDL oxidation.

Poster Session

OXIDATION OF CYANIDE TO CYANATE BY MYELOPEROXIDASE: A NEW ROUTE FOR APOLIPOPROTEIN CARBAMYLATION?

Pierre Van Antwerpen[1], K. Zouaoui Boudjeltia[2], P. Furmueller[3], M. Dieu[4], C. Delporte[5], M. Raes[4], N. Moguilevsky[6], M. Vanhaeverbeek[2], J. Ducobu[2], J. Nève[5], C. Obinger[3]

[1]Analytical Platform of the Institute of Pharmacy, Universite Libre de Bruxelles, Brussels, [2]Laboratory of Experimental Medicine, CHU Charleroi, Hopital A. Vésale, Université Libre de Bruxelles, Montigny-le-Tilleul, Belgium, [3]Department of Chemistry, Division of Biochemistry, University of Natural Ressources and Applied Life Sciences (BOKU), Vienna, Austria, [4]Laboratory of Cellular Biology, University of Namur (FUNDP), Namur, [5]Laboratory of Pharmaceutical Chemistry, Institute of Pharmacy, Universite Libre de Bruxelles, Brussels, [6]Technology Transfer Office, University of Namur (FUNDP), Namur, Belgium

Protein carbamylation by cyanate is a post-translational modification correlated with several (patho)physiological conditions. The present study aims to describe further pathways of carbamoyl formation that involve myeloperoxidase (MPO). It demonstrates that MPO is able to catalyze the two-electron oxidation of cyanide to cyanate thereby promoting the carbamylation of lysine residues of apolipoprotein B100. In addition two further pathways of protein carbamylation are shown that involve two main reaction products of MPO, namely hypochlorous acid (HOCl) and chloramines.

The release of ^{13}C-cyanate derived from ^{13}C-cyanide oxidation by MPO has been probed by tandem-quad mass spectrometry. A product with an appropriate mass-to-charge ratio (*m/z*=43) was obtained with a fragment-ion at 27 *m/z*. Upon using [^{13}C]-CN or [^{13}C,^{15}N]-CN, peaks at *m/z* values of 43.003 or 44.000 were found with a high-resolution mass spectrometer (QTOF) suggesting formation of the isotopomers of cyanate. After addition of lysine to the MPO/H_2O_2/cyanide (and its isotopomers) system and analysis of the reaction products, high-resolution peaks corresponding to isotopomers of carbamyllysine (homocitrulline) were found. This reaction was also performed with ApoB100 of LDL and the acid hydrolysis demonstrated the presence of homocitrulline. Finally, it was also demonstrated that HOCl mediates the two-electron oxidation of cyanide to cyanate and that monochloramine mixed with [^{12}C]- or [^{13}C]thiocyanate is able to promote carbamylation reactions on ApoB100.

MPO therefore promotes carbamylation reactions in four ways:

(i) by oxidation of thiocyanate[1],

(ii) by direct oxidation of cyanide,

(iii) by reaction of hypochlorous acid with cyanide, or (iv) by reaction of chloramines with thiocyanate.

Poster Session

INVESTIGATION OF THE SUBSTRATE- AND STEREOSELECTIVITY OF ADIPOSE TRIGLYCERIDE LIPASE

Thomas Oliver Eichmann, A. Lass, R. Zimmermann, R. Zechner

Institute of Molecular Biosciences, University of Graz, Graz, Austria

Diacylglycerols (DAG) and free fatty acids (FFA) are intermediary products in lipid metabolism. More recently, they have been recognized as important signaling molecules activating protein kinase C (PKC) and specific classes of nuclear receptors, respectively. DAG and FFA may derive from lipolytic breakdown of phospholipids and triacylglycerol (TAG). DAG, constitute chiral molecules with three different stereospecific conformations (*sn*-1,2 DAG, *sn*-2,3 DAG and *sn*-1,3 DAG). FFA occur physiologically as wide ranging group consisting of species different in chain-length and saturation grade.

In this study, we investigated the conformation of DAG as well as FFA species derived from adipose triglyceride lipase (ATGL)-mediated hydrolysis of different TAG substrates. Our results suggest that ATGL specifically hydrolyzes the ester bond at the second carbon atom of TAG leading to the formation of *sn*-1,3 DAG. Furthermore, ATGL stimulated by its coactivator CGI-58 hydrolyzes the ester bond at the first or second carbon atom resulting in the formation of *sn*-2,3 DAG and *sn*-1,3 DAG, respectively. GC/MS analysis of FFA released from fat pads of wildtype, hormone-sensitive lipase (HSL)- and ATGL-knockout mice indicate that ATGL catalyzes predominantly the cleavage of unsaturated FFA.

Together, our data suggest that ATGL hydrolyzes TAG in a stereospecific as well as fatty acid species specific manner. Thus, we speculate that these ATGL-derived lipolytic intermediates may effect a variety of cellular signaling pathways.

Poster Session

IDENTIFICATION AND CLONING OF NOVEL SOLUBLE AND MEMBRANE BOUND FORMS OF HUMAN ATP-BINDING CASSETTE TRANSPORTER G4

Yoshinari Uehara, S. Abe, E. Yahiro, A. Kawamura, T. Iwamoto, S.-I. Miura, K. Saku

Fukuoka University, Fukuoka, Japan

ATP-binding cassette transporter G4 (ABCG4), a membrane cholesterol transporter has been implicated to mediate cholesterol efflux in the presence of HDL as ABCG1. However, the physiological function of ABCG4 has not been established. Our previous report shows the microglial ABCG4 has been upregulated in Alzheimer´s disease (AD) brain. ApoA-I-, apoE- and HDL-mediated cholesterol efflux were significantly increased in human full-length ABCG4 (2.0kb) transfected cells. Since there was large discrepancy in the molecular weight of ABCG4 protein as compared with previous report, we performed to analyze the isoform of ABCG4. The mRNAs were extracted from AD brain, and ABCG4 isoforms were cloned using rapid amplification of cDNA ends (RACE) technique. Here we report the cloning of a novel isoform of human ABCG4. 5´-RACE identified an upstream exon and 3 kinds of splicing, not deduced from known sequences of ABCG4. 3´-RACE also identified an exon not deduced from known sequences of ABCG4, and it was short-isoform of ABCG4 (sABCG4) which was only half size (1.1kb) of full-length ABCG4. Examination of the expression of sABCG4 mRNA revealed that sABCG4 mRNA was also expressed in macrophage by RT-PCR. sABCG4 transfected cells expressed sABCG4 proteins with both soluble and membrane bound forms. The sABCG4 is possible to regulate HDL metabolism to interact with other lipid transporters, in addition soluble ABCG4 might be a novel marker for AD and cardiovascular diseases.

Poster Session

GENETIC VARIATION IN THE ABCA1-GENE ABROGATES THE PROTECTIVE EFFECT OF ADIPONECTIN ON CAROTID IMT

Christian Ciardi[1], A. Sandhofer[1], B. Iglseder[2], B. Paulweber[3], J. Patsch[1]

[1]Department of Internal Medicine I, Medical University Innsbruck, Innsbruck, [2]Department of Geriatrics, [3]First Department of Internal Medicine, Paracelsus Private Medical University Salzburg, Salzburg, Austria

Introduction: The ATP-binding cassette transporter A1 (ABCA1) facilitates amongst others the initial step of the reverse cholesterol transport by transporting cholesterol from macrophages to lipid-poor HDL precursors. Variants in the ABCA1-gene have been associated with a lower carotid intima media thickness (IMT). Adiponectin levels are correlated negatively with carotid IMT and positively with HDL-C. Recent in vitro data suggested an influence of adiponectin on ABCA1-mediated cholesterol efflux from macrophages.

Objective: Our objective was to investigate the R219K polymorphism of the ABCA1 gene influences the atheroprotective effect of adiponectin.

Design: 680 men randomly were selected from the SAPHIR-population.

Methods: The R219K-variant was determined by RFLP-analysis, carotid IMT by high-resolution B-mode ultrasound and plasma adiponectin levels by ELISA.

Results: In carriers of the K-variant (n=334) adiponectin shows a negative correlation with carotid IMT ($r=-0.137$, $p=0.013$), whereas in wild-type subjects (n=346) homozygous for the R allele no correlation can be detected ($r=-0.044$, $p=0.42$). In carriers this atheroprotective effect persisted after adjustment for age, blood pressure, LDL-cholesterol, C-reactive protein and insulin resistance (standardized Beta = -0.129, $p=0.009$). Plasma adiponectin levels correlated positively with HDL-C in the whole study population ($r=0.337$, $p< 0.001$), in wildtype subjects ($r=0.312$, $p< 0.001$) and in carriers of the K-variant ($r=0.387$, $p< 0.001$). However, adjustment for HDL-cholesterol abrogates the correlation of IMT and adiponectin in the whole population, wild-type subjects and carriers of the K-variant.

Conclusion: Variation in the ABCA1-gene influences the atheroprotective effect of adiponectin, suggesting that adiponectin influences carotid IMT at least partly via ABCA1.

Poster Session

NEW SPLICING MUTATIONS OF MTP LEADING TO SEVERE ABETALIPOPROTEINEMIA

E. Mas[1,2], V. Pons[2], C. Rolland[2], M. Nauze[2], M. Danjoux[3], G. Gaibelet[2], A. Sassolas[4], E. Lévy[5], F. Tercé[2], **Xavier Collet**[2]

[1]Unité de Gastroentérologie, Hépatologie et Nutrition - Département de Pédiatrie - Hôpital des Enfants, [2]Inserm U563; CPTP, IFR 150, [3]Département d'Anatomopatholgie, Hôpital Purpan Purpan, Toulouse, [4]Laboratoire des Dyslipidémies, Centre de Biologie Est, Lyon, France, [5]Départements de Nutrition et de Biochimie, Hôpital Sainte-Justine et Université de Montréal, Montréal, QC, Canada

Background: Abetalipoproteinemia (ABL) is a rare autosomal recessive disease characterized by very low plasma levels of cholesterol and triglycerides (TG) and the absence of apo B-containing lipoproteins. It is the consequence of microsomal triglyceride transfer protein (MTP) deficiency.

Methods: We reported 2 new patients with a severe form of ABL. We determined MTP mutations and we analysed their functional involvement in MTP function.

Results: These children have the intestinal and biochemical characteristics of ABL including duodenal fat storage and absence of chylomicrons and LDL lipoproteins. We confirmed that they are compound heterozygotes with 619 G>T and c.1237-28 A>G mutations. cDNA analysis revealed alternative splicing with deletion of exon 6 and 10, respectively. Deletion of exon 6 introduced a frame shift in the reading frame and a premature stop codon at position 234; the truncated protein was not translated. Although Δ10-MTP was normally present at the endoplasmic reticulum (ER), there was no transfer of TG using duodenal biopsies.

Conclusion: Amino acids 413-448 are required for MTP TG transfer but their loss does not impair the localization of MTP at the ER.

Poster Session

FUNCTION OF HDL MODIFYING PROTEINS IN THE HUMAN PLACENTA

Jani Saarela[1], J. Metso[1], R. Kaaja[2], W.J. Schneider[3], M. Jauhiainen[1]

[1]Public Health Genomics Unit, National Institute for Health and Welfare, [2]Department of Obstetrics and Gynaecology, University of Helsinki, Helsinki, Finland, [3]Department of Medical Biochemistry, Medical University of Vienna/MFPL, Vienna, Austria

Object of the study: Pre-eclampsia is a common disorder during pregnancy and a serious threat for both the mother and the baby. Furthermore, it predisposes both the mother and the baby later in life to cardiovascular diseases. In the pre-eclamptic placenta many changes already occur during early pregnancy and the placenta becomes greasy due to dysfunctional lipid-metabolism. The reason why pre-eclampsia develops is unknown and predictive markers are lacking. We aimed to decipher how HDL modifying proteins (e.g. PLTP, CETP, ABCA1, ABCG1, SRBI) participate in the transfer of lipids from the maternal to the embryonic circulation and whether any of them can be used as predictive markers of the disorder.

Methods: Measurement of human placenta PLTP- and CETP-activity (radiometric methods), PLTP-mass (ELISA), Western-blotting, qRT-PCR, and lipid measurements to determine fattening of the placenta. As cell model, we are using human placenta derived BeWo-cells and aim to study regulation of LXR/RXR/FXR target genes and the effect of estrogen and progesterone in the context of HDL metabolism.

Results: Our preliminary results show that in type-1-diabetes placenta PLTP activity is increased (39%) and in pre-eclampsia placenta reduced (37%) compared to control placenta. In addition, in diabetic placenta phospholipid levels are increased (55%). Properties of the BeWo-cell derived PLTP are similar to plasma PLTP.

Conclusions: The majority (44%) of the umbilical cord cholesterol is carried by HDL-particles. PLTP, CETP, ABCA1, ABCG1, and SRBI are highly expressed in human placenta. The preliminary results suggest that HDL modifying factors are important in maternal-fetal lipid transport.

Poster Session

EZETIMIBE EFFECT ON SERUM APOB48 LEVELS IN FAMILIAL HYPERCHOLESTEROLEMIA AND TYPE 2 DIABETES

Mariko Sudo, S. Takemitsu, Y. Sato, M. Nagao, T. Harada, Y. Nakajima, K. Tanimura, F. Okajima, S. Oikawa

Nippon Medical School, Tokyo, Japan

Background and aims: Chylomicron synthesis followed by the assembly with apoB48 will be increased in diabetic condition or insulin resistant state. Ezetimibe is a unique agent binding to NPC1L1 molecule of enterocyte plasma membrane and inhibits cholesterol absorption. On the other hand cholesterol absorption will be increased in diabetes or insulin resistant state through NPC1L1 function. The focus of the present study is on the changes of serum lipids and apoB48 levels after monotherapy with Ezetimibe for 3-4 months in hypercholesterolemia patients.

Materials and methods: Subjects were FH (n=30, 57±11 years old, mean±SD), Type 2 diabetes (DM, n=33, 58±11), nonDMnonFH (n=22, 56±11). Serum lipids and apoB48 levels were studied before and after Ezetimibe monotherapy (10 mg/day) during 3-4 months.

Results: Ezetimibe significantly decreased serum TC, LDL-C and non-HDL-C levels in each group. The %changes of LDL-C were higher in DM (-22%) compared to FH (14%) and nonDMnonFH (-15%). The significant changes of serum TG and apoB48 levels were observed in DM (-10% and 6%, respectively), but not in FH or nonDMnonFH. It was suggested that the decrease of apoB48 after treatment with Ezetimibe was related to TG reduction.

Conclusion: Inhibition of cholesterol absorption by Ezetimibe will be more effective in DM compared to nonDM subjects. Ezetimibe effect on apoB48 will be affected through chylomicron metabolism.

Poster Session

RESEQUENCING THE LPL GENE IN PATIENTS WITH VARIOUS HYPERTRIGLYCERIDEMIAS TO DETERMINE THE RELATIVE IMPORTANCE OF RARE ALLELES AND COMMON VARIANTS

D. Evans, J. Azar, **Frank Beil**

Endokrinologie und Stoffwechsel, Med. Klinik III, UKE, Hamburg, Germany

Common SNPs in the LPL gene have been shown to have a modest affect on triglyceride levels in a number of studies whereas resequencing studies in patients with severe hypertriglyceridemia (>10mmol/l) have shown that rare mutations are frequent. It is the aim of this study to determine the role of rare mutations in the LPL gene in patients with moderate hypertriglyceridemia. The exons plus exon/intron boundaries of the LPL gene of three groups of patients were sequenced: Group A. 97 patients with severe hypertriglyceridemia defined as triglycerides above 10mmol/l. Group B, 220 patients with triglycerides above the 95th percentile for age and sex but below 10mmol/l. Group C 120 patients with APOE2/2 genotype. In addition to known SNPs, 18 rare variants were identified, 5 of which have been previously reported (V69L,5x, G188E 3x, I194T 1x, I225T, 2x and c440-443delACTA 1x). New variants identified were: M-36L, R89W, Y94S, I196F, Y206D, Y262X, S266P, S277N, C278T, M301I and E347D. In group A 12 patients were carriers of at least one rare variant and 11 were carriers of the N291S SNP. In group B, there were 9 carriers of rare variants plus 19 N291S and 2 D9N carriers. In group C there were 2 rare variant carriers plus 3 D9N and 6 N291S. In conclusion in Type III HLP neither rare nor common variants play a significant role whereas both types are equally important in severe hypertriglyceridemia, in moderate hypertriglyceridemia rare variants are found but common variants are more frequent.

Poster Session

HYPOGLYCEMIC POTENTIAL OF *CUCURBITA PEPO* L. IN ALLOXAN-INDUCED DIABETIC RATS

Sedigheh Asgary[1], A. Adelnia[1], S. Kazemi[1], F. Shamsi[1], M. Rafieian-Kopaei[2], Asgary, S

[1]Applied Physiology Research Center, Isfahan, [2]Medicinal Plant Research Center, Shahrekord, Iran

Background: Diabetes mellitus is a metabolic disorder which resulting from perturbation in insulin secretion (type 1) or insulin action (type 2). Chronic hyperglycemia cause complication linked to diabetes, such as atherosclerotic vascular disease. Phytochemicals identified from medicinal plants are presenting an exciting opportunity for the development of new types of therapeutic in diabetes mellitus. Pumpkin (*Cucurbita pepo* L.) is one of those promising plants. The aim of the present study was to determine the antihyperglycemic effect of powder of pumpkin on level of some biochemical parameters in diabetic rats. The therapeutic effect of the plant was compared with a reference drug, Glibenclamide.

Methods: Diabetes mellitus was induced by a single intraperitoneal (IP) injection of Alloxan (120 mg/Kg BW). 28 male Wistar rats were divided into 4 groups of 7 each. The groups included nondiabetic, diabetic with no treatment, diabetic rats treated with pumpkin powder (1 g/kg BW) by gastric intubation and diabetic rats treated with Glibenclamide (0.6 mg/kg BW) by IP injection. Diabetic rats were treated daily for 4 weeks.

Result: Diabetic rats exhibited an increase in level of fasting blood glucose, cholesterol, triglyceride, LDL and decrease in HDL level compared to nondiabetic rats. Treatment of diabetic rats with extract and glibenclamide could restore the change of the above parameters to their normal levels.

Conclusion: The observations of this study indicate that phytochemicals present in *Cucurbita pepo* may play a therapeutic role in combating diabetes and diabetes-related complication.

Keywords: Pumpkin, diabetes, alloxan, *Cucurbita pepo.*

Poster Session

CLASSIFICATION OF DYSLIPIDEMIAS BASED ON APOLIPOPROTEIN B AND TRIGLYCERIDES. ASSOCIATION OF DYSLIPIDEMIC PHENOTYPES WITH MARKERS OF INSULIN RESISTANCE AND INFLAMMATION

Helena Vaverkova[1], D. Karasek[2], D. Jackuliakova[2], D. Novotny[3], M. Halenka[2], J. Lukes[3], L. Slavik[4]

[1]3rd Department of Internal Medicine, Medical Faculty of University Palacký and University Hospital Olomouc, [2]3rd Department of Internal Medicine, [3]Department of Clinical Biochemistry, [4]Department of Hematooncology, University Hospital Olomouc, Olomouc, Czech Republic

Growing evidence suggests that apolipoprotein B (ApoB) is a better marker of cardiovascular risk than LDL-C. Recently, Sniderman suggested a new classification of dyslipidemias into 4 dyslipidemic phenotypes (DLP) based on ApoB and triglycerides (TG): DLP 1: ApoB< 1.2 g/l and TG< 1.5 mmol/l; DLP 2: ApoB< 1.2 and TG≥1.5; DLP 3: ApoB≥1.2 and TG< 1.5; DLP 4: ApoB≥1.2 and TG≥1.5.

The aim of our study was to compare association of LDL-C and ApoB with other risk factors for atherosclerosis and evaluate association of above mentioned DLP with lipids, markers of insulin resistance and inflammation.

Material and methods: We examined 348 patients of our lipid clinic (not treated with hypolipidemic drugs).

Results: Both LDL-C and ApoB correlated positively with TC, TG, non-HDL-C, and Lp(a). Furthermore, ApoB (but not LDL-C) correlated with BMI, waist, SBP, DBP, insulin, HOMA, C-peptide, proinsulin, vWF, tPA, PAI-1, sICAM-1, hsCRP ($p< 0.05$-0.001 for all parameters). ApoB/ApoA-1 ratio and non-HDL-C increased from DLP 1 to DLP 4 ($p< 0.001$ for both parameters). Hypertriglyceridemic DLP (2 and 4) had in comparison with DLP 1 increased SBP, DBP, markers of insulin resistance and sICAM-1 ($p< 0.05$-0.001 for all parameters).

Conclusion: Our results support ApoB as a better marker of risk than LDL-C. Increased triglyceride levels are good markers of insulin resistance. ApoB/ApoA-1 ratio and non-HDL-C as best lipid markers of cardiovascular risk increase from DLP 1 to DLP 4. Thus, above mentioned classification seems to have a good rationalization.

Supported by grant IGA MZ CR NS/10284

Poster Session

ASPIRIN'S EFFECT ON OXIDATIVE STRESS IN PATIENTS WITH TYPE II DIABETES MELLITUS

J. Geske[1], K. Guyer[1], I. Muncy[1], M. Boisen[1], I. Garcia De La Torre[2], **Luis Lopez**[1]

[1]Corgenix, Inc, Broomfield, CO, USA, [2]Immunology and Rheumatology, Hospital General de Occidente, Zapopan, Mexico

Introduction: Chronic platelet activation and lipid peroxidation are two factors associated with atherosclerosis that promote the production of arachidonic acid metabolites. Inhibition of arachidonic acid-derived thromboxane A2 is the main objective of aspirin (ASA) treatment in primary and secondary prevention of CVD.

Objectives: To evaluate whether diabetes mellitus (DM) patients demonstrate high thromboxane A2 baseline levels that may impair their response to ASA and whether ASA in DM patients reduces oxidative stress.

Results: We measured urinary 11dhTxB2, a marker of systemic thromboxane production and 8-iso PGF2α, a marker of in vivo oxidative stress in 73 type II DM patients and 25 apparently healthy individuals before and after receiving either 325mg/100mg of ASA daily for one week. Median 11dhTxB2 levels post aspirin ingestion were significantly decreased for both DM and control groups 3141 vs. 699 pg/mg creatinine ($p< 0.001$) and 2113 vs. 431 pg/mg creatinine ($p< 0.001$) respectively. Median 11dhTxB2 level before ASA was significantly higher in the DM group vs. control 3141 vs 2113 pg/mg creatinine ($p=0.025$). The median 8-iso PGF2α level post ASA ingestion was not significantly changed for either the DM or the control group 1384 vs. 1533 pg/mg creatinine, and 1221 vs. 1260 pg/mg creatinine, respectively.

Conclusion: Elevated 11dhTxB2 suggests an increased platelet activation state in DM that is modifiable by ASA. However, ASA though ASA significantly reduces thromboxane levels in both DM patients and controls, it has no apparent effect on oxidative stress assessed by 8-iso PGF2α.

Poster Session

ABCG5 POLYMORPHISMS MODULATE PLASMA LIPID LEVELS: INSIGHT FROM THE SPANISH FAMILIAL HYPERCHOLESTEROLEMIA COHORT STUDY

Antonio Garcia Rios[1], P. Perez Martinez[1], P. Mata Lopez[2], F. Fuentes Jimenez[1], R. Alonso Karlezi[2], J. Caballero[1], N. Mata[2], J.M. Ordovas[3], F. Perez Jimenez[1], J. Lopez Miranda[1]

[1]Hospital Universitario Reina Sofia, Cordoba, [2]Fundacion Jimenez Diaz, Madrid, Spain, [3]Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA

Background: Familial hypercholesterolemia is caused by mutations in the gene encoding the low-density lipoprotein receptor. It is well known that the phenotypic and clinical expression of cardiovascular disease in these patients is highly variable in terms of the age of onset and severity, even for those sharing the same mutation. Despite the importance of *ABCG5/G8* transporters in the regulation of cholesterol kinetics in humans, no study has related human *ABCG5/G8* gene polymorphisms and plasma lipids concentrations in patients with familial hypercholesterolemia (FH).

Objective: To investigate the association of four common single nucleotide polymorphisms (SNPs) at *ABCG5* (i7892A>G, i18429C>T, Gln604GluC>G, i11836G>A) and five at *ABCG8* (5U145T>G, Tyr54CysA>G, Asp19HisG>C, i14222T>C, and Thr400LysG>T) with plasma lipids concentrations.

Methods: Nine *ABCG5/G8* SNPs were genotyped in 465 subjects with genetic diagnosis of FH who were included in the Spanish National FH cohort.

Results: Carriers of the minor A allele at the *ABCG5*_i11836G>A SNP displayed significantly higher HDL-C concentrations (*P*=0.023) than G/G subjects. In addition, carriers of the minor G allele at the *ABCG5*_Gln604GluC>G SNP had significantly lower VLDL-C (*P*=0.011) and lower TG (*P*=0.017) concentrations than homozygous for the mayor C allele. No other significant associations were found between all these SNPs explored and other plasma lipid variables.

Conclusion: This study demonstrates that ABCG5 genetic variants modulate plasma lipids concentrations in patients with familial hypercholesterolemia. These results could explain the differences in the susceptibility to coronary heart disease in these patients.

"**Funding:** CIBER CBO/6/03, Instituto de Salud Carlos III".

Poster Session

PERTURBED CELLULAR LRP1 LOCALIZATION LEADS TO INCREASED HEPATIC LDLR EXPRESSION WITH ATHEROPROTECTIVE CONSEQUENCES

Philip Gordts[1], A. Bartelt[2], S. Nilsson[3], W. Annaert[4], J. Heeren[2], A. Roebroek[1]

[1]Laboratory for Experimental Mouse Genetics, Department of Human Genetics, KU Leuven, Leuven, Belgium, [2]IBMII: Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Division of Physiological Chemistry, Department of Medical Biosciences, Umeå University, Umeå, Sweden, [4]Laboratory of Membrane Trafficking, Department of Human Genetics, KU Leuven, Leuven, Belgium

Objectives: The intracellular domain of LRP1 contains a number of potential signaling motifs which seem to be relevant for lipoprotein transport and development of atherosclerosis. By analysis of knock-in mutations into the intracellular domain of endogenous murine LRP1 we wanted to identify the in vivo relevance of these motifs.

Methods: Knock-in mice carrying an inactivating mutation in the NPxYxxL motif were crossed with $ApoE^{-/-}$ mice in order to evaluate the impact on lipid metabolism and atherosclerosis.

Results: On the $ApoE^{-/-}$ background the inactivation of NPxYxxL motif was associated with a significant decrease in CR and VLDL levels. Hepatic VLDL production, intestinal lipid absorption and production seem not to be affected; however, postprandial lipid clearance was accelerated. A first set of experiments seem to indicate that inactivation of the NPxYxxL motif reduces the presence of LRP1 in endosomal/recycling compartments and also impaired the insulin mediated translocation of LRP1 to the plasma membrane in hepatocytes. These impairments were associated with a compensatory increase in LDLR protein levels in hepatocytes and a 1.5-fold increase in CR uptake. The impact of the LRP1 NPxYxxL inactivation on atherosclerosis was evaluated in 1 year-old mice and showed a substantially diminished plaque surface.

Conclusion: The distal NPxYxxL motif of LRP1 is important for its cellular distribution. The data suggest that impaired LRP1 localization leads to a compensatory increase in hepatic LDLR with an improved lipid profile as a consequence. Most importantly manipulation of the LRP1 translocation might serve an atheroprotective endpoint.

Poster Session

THE EFFECTS OF HYPOLIPIDEMIC TREATMENT ON LIPOPROTEIN-ASSOCIATED PHOSFHOLIPASE A2 IN PATIENTS WITH MIXED HYPERLIPIDEMIA

Aris P Agouridis[1], P.S. Kazakou[1], T.D. Filippatos[1], C.C. Tellis[2], M.S. Elisaf[1], A.D. Tselepis[2]

[1]Internal Medicine, Medical School, University of Ioannina, [2]Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece

Background: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is considered as an emerging cardiovascular risk factor and is commonly encountered in subjects with mixed hyperlipidemia.

Objective: We investigated the effects of rosuvastatin, alone or in combination with fenofibrate or ω-3 fatty acids on plasma Lp-PLA2 activity and mass in patients with mixed hyperlipidemia.

Methods: Patients with mixed hyperlipidemia received for 3 months rosuvastatin 40 mg/day (n=25, ROS group) or rosuvastatin 10 mg/day plus fenofibrate 200 mg/day (n=20, ROSFE group) or rosuvastatin 10 mg/day plus 2gr of ω-3 fatty acids (n=25, ROSOM group). At baseline and after 3 months of treatment the lipid profile and plasma Lp-PLA2 activity and mass were determined.

Results: In all treatment groups a significant reduction of total plasma Lp-PLA2 activity was observed: In ROS group by 39.3%, $p< 0.001$, in ROSFE group by 27.3%, $p< 0.001$ and in ROSOM group by 26.7%±17.4, $p< 0.001$. The reduction of Lp-PLA2 activity was greater in the ROS group ($p=0.037$ compared with ROSFE and $p=0.017$ compared with ROSOM). The Lp-PLA2 mass was reduced more in the ROS group by 26.4% and also in the ROSOM group by 22.5% [$p=0.003$ and $p=0.024$ compared with ROSFE group (13.7% reduction), respectively]

Conclusions: High doses of rosuvastatin, small doses of rosuvastatin plus fenofibrate and small doses of rosuvastatin plus ω-3 fatty acids exhibited favorable effects on Lp-PLA2 activity and mass. However, rosuvastatin monotherapy had a more potent effect on Lp-PLA2 activity compared with rosuvastatin plus fenofibrate or rosuvastatin plus ω-3 fatty acids in patients with mixed hyperlipidemia.

Poster Session

DETERMINATION OF VLDL CHOLESTEROL: IS THERE ANYTHING BETTER THAN THE FRIEDEWALD TERM?

Michaela Vossen[1], E. Vettorazzi[2], K. Wegscheider[2], A. Laatsch[1]

[1]Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, [2]Department of Medical Biometry and Epidemiology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Background: Plasma lipoprotein concentrations are clinically important for the treatment and diagnosis of dyslipidemia associated with cardiovascular diseases. Commonly, HDL cholesterol is measured directly, while LDL cholesterol is calculated with the Friedewald formula by estimating VLDL cholesterol (VLDL-C). This work aims at deriving a more accurate formula to reliably calculate VLDL-C.

Method: The cholesterol content of different lipoprotein fractions can be measured after separation by FPLC. Such direct data from a cohort of 91 healthy subjects were used to derive an approximation for VLDL-C. The derived formula was then tested in 48 volunteers.

Results: VLDL-C calculated from the common estimation of mass concentration being TAG/5 differed systematically from FPLC-derived VLDL-C values. Therefore, we developed a mathematical formula to calculate VLDL-C very accurately. This formula was subsequently simplified to be easily applicable to all metabolic situations. This formula is VLDL-C [mg/dL] = TAG/10 - 3 for TAG values smaller than 400mg/dl. It yielded values highly correlating with FPLC-derived VLDL-C. The differences between this formula and the FPLC measurement were clinically negligible when applied to an independent cohort of 48 volunteers. In addition, this formula yielded accurate concentrations for postprandial triacylglyceride-rich lipoprotein cholesterol (TRL-C) when applied in the first cohort of 91 subjects after a high-fat breakfast as well as an oral glucose tolerance test.

Conclusions: The newly developed formula for fasting VLDL-C and postprandial TRL-C is an easy and accurate way to approximate VLDL-C and postprandial TRL-C, respectively, which might be important to detect the cardiovascular risk connected to hypertriacylglyceridemia.

Poster Session

THE INHIBITION OF UBIQUITIN-PROTEASOME-DEPENDENT DEGRADATION OF ABCA1 AND ABCG1 ENHANCES REVERSE CHOLESTEROL TRANSPORT FROM MACROPHAGES IN VITRO AND IN VIVO

Masatsune Ogura, M. Ayaori, H. Kondo, K. Ikewaki

Internal Medicine, National Defense Medical College, Etajima, Japan

Background and aim: Our objective was to investigate whether ubiquitin-proteasome pathway plays a role in ATP-binding cassette transporter (ABC) A1 and ABCG1 expression, and reverse cholesterol transport (RCT) involving macrophages in vitro and in vivo.

Methods and results: Treatment with various proteasome inhibitors, MG132, epoxomicin and bortezomib, increased ABCA1 and ABCG1 expression in THP-1 macrophages and RAW264.7 cells, translating into enhanced apoA-I- and HDL-mediated cholesterol efflux from the macrophages. Pulse-chase analysis with ^{35}S-methionine/cysteine revealed that treatment with MG132 resulted in inhibition of ABCA1 and ABCG1 protein degradation. We demonstrated that both ABCA1 and ABCG1 proteins undergo polyubiquitination in THP-1 macrophages and HEK293 cells transfected with the expression vectors. Furthermore, we performed in vivo experiments. It revealed that ABCA1 and ABCG1 protein levels were increased in peritoneal macrophages from mice treated with bortezomib compared with the control animals. To further assess RCT in vivo, ^{3}H-cholesterol-labeled and acetyl LDL-loaded RAW264.7 cells were intraperitoneally injected into mice and appearance of ^{3}H-tracer in plasma, liver and feces was monitored. Supporting in vitro data, bortezomib treatment significantly increased the levels of ^{3}H-tracer in both plasma and feces.

Conclusion: These findings indicate that the ubiquitin-proteasome system is involved in ABCA1/ABCG1 degradation and RCT in vivo. Enhancing RCT by specific inhibition of these pathways might be promising therapeutic strategies for atherosclerosis.

Poster Session

APOE*3LEIDEN.CETP TRANSGENIC MICE AS MODEL FOR THE METABOLIC SYNDROME

José WA van der Hoorn[1,2], A.M. van den Hoek[1,2], A.C. Maas[1], R.M. van den Hoogen[1], A. van Nieuwkoop[1], E.O. Offerman[1], L.M. Havekes[1,2], H.M. Princen[1]

[1]Vascular and Metabolic Diseases, TNO BioSciences, [2]Endocrinology, Leiden University Medical Center, Leiden, The Netherlands

Background: The metabolic syndrome is characterized by the co-occurrence of several risk factors i.e. increased body weight (bw) and insulin resistance (IR) and at the same time adverse changes in plasma lipids as observed in diabetic dyslipidemia, with increased triglycerides and apoB-containing lipoproteins and decreased HDL.

The aim of this study was to investigate whether the APOE*3Leiden.CETP (E3L.CETP) mouse is a useful translational animal model to investigate the metabolic syndrome.

Methods: Male E3L.CETP mice were put on a high fat diet and fructose in drinking water for 12 weeks to induce diet-induced obesity and IR. Then the mice were treated with either rosiglitazone (3 and 11 mg/kg bw/d), resveratrol (75 mg/kg bw/d), fenofibrate 12 mg/kg bw/d), atorvastatin 10 mg/kg bw/d) or niacin 720 mg/kg bw/d) for 4 weeks.

The effects on bw, plasma lipid and inflammation parameters and insulin sensitivity (via hyperinsulinemic euglycemic clamps) were assessed.

Results: Dietary treatment resulted in a human-like lipoprotein profile with a TC/

HDL-C ratio of 3. Anti-diabetic compound rosiglitazone significantly increased insulin sensitivity and reduced plasma lipid levels. Established lipid lowering compounds atorvastatin, fenofibrate and niacin, and resveratrol improved the dyslipidemia.

Conclusion: The data indicate that the E3L.CETP mouse is a good translational animal model that combines all important features that underlie the metabolic syndrome and mimic the human response to clinically used treatments. We conclude that the E3L.CETP mouse is a promising model to investigate the effects of new drugs, alone or in combination, that affect insulin resistance and diabetic dyslipidemia.

Poster Session

HIGH SERUM LIPOPROTEIN(A) IS MORE STRONGLY ASSOCIATED WITH PERIPHERAL ARTERIAL DISEASE THAN WITH STABLE CORONARY ARTERY DISEASE

Alexander Vonbank[1,2,3], C.H. Saely[1,2,3], P. Rein[1,2,3], S. Beer[1,2,3], H. Drexel[1,2,3,4]

[1]VIVIT Institute, [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Objectives: Lipoprotein a (Lp(a)) is a well established risk factor for cardiovascular disease. It is unkown whether high Lp(a) is more strongly associated with coronary artery disease (CAD) or with peripheral arterial disease (PAD).

Methods: We measured serum Lp(a) in 621 consecutive patients with angiographiaclly proven stable CAD as well as in 193 consecutive Patients with sonographically proven PAD.

Results: Median [interquartile range] Lp(a) was significantly higher in PAD patients than in CAD patients (21 vs. 14.5; $p < 0.001$). Adjustment for age, gender, diabetes, hypertension and smoking status confirmed that high Lp(a) was significantly stronger associated with PAD than with CAD.

Conclusion: High Lp(a) is more strongly associated with PAD than with stable CAD.

Poster Session

AN APOJ DECAPEPTIDE REGULATES MONOCYTE ADHESION TO ENDOTHELIAL CELL MONOLAYERS

Kaveh Navab, L. Vakili, S. Safarpoor, N. Kamranpour, O. Elboudwarej, M. Garife, S. Hama, G. Hough

David Geffen School of Medicine, UCLA, Los Angeles, CA, USA

Inflammation is involved in numerous pathological conditions and monocyte-endothelial interaction is central in the etiology inflammation. We have examined the effect of a decapeptide on the adhesion of monocytes to endothelial cells.

Methods: The peptide Ac-L-V-G-R-Q-L-E-E-F-L-NH_2 was made based on the sequence of apoJ using a standard solid phase synthesis protocol and was used at 1.0 microgram per ml of the solution used. Human monocytes were isolated from the blood of consented healthy donors in our core using modified Recalde method. Human aortic endothelial cells were isolated from the not needed trimmings of the aorta following the use of donor heart for transplantation. Endothelial cells were plated and grown to confluence. Monocytes were incubated with the peptide for an hour at 37 degrees C followed by washing and addition to endothelial monolayers. Following 15 minutes of incubation at 37degrees C, the monolayers were washed to remove the loosely bound monocytes and the number of bound monocytes were microscopically determined in six high power fields.

Results: Incubation of the monocytes with the decapeptide vs. a scrambled version of the peptide, resulted in a significant decrease in the adhesion of monocytes to endothelial monolayers (12.5+/- 1.1 vs. 18.2+/ 1.6 monocytes per high power filed, $p< 0.01$).

Conclusion: The decapeptide Ac-L-V-G-R-Q-L-E-E-F-L-NH_2 is capable of reducing monocyte adhesion to endothelial cells and might be effective in preventing increased monocyte endothelial interactions in inflammatory settings.

Poster Session

ANGIOPOIETIN-LIKE PROTEIN 4 (ANGPTL4) SERUM LEVELS ARE INVERSELY CORRELATED WITH OBESITY IN YOUNG ADULTS

Marius Robert Robciuc[1], K.H. Pietiläinen[2], N. Lundbom[3], A. Hakkarainen[3], J. Kaprio[1,4], A. Rissanen[2], M. Jauhiainen[1], C. Ehnholm[1]

[1]National Institute for Health and Welfare and Finnish Institute for Molecular Medicine, [2]Obesity Research Unit, Helsinki University Central Hospital, [3]Helsinki Medical Imaging Centre, University of Helsinki, [4]Department of Public Health, University of Helsinki, Helsinki, Finland

Animal studies suggest a regulatory role for Angptl4 in obesity. The aim of the study was to investigate possible associations between serum Angptl4 and obesity related parameters in humans.

Serum Angptl4 levels were measured by ELISA in two population samples: 1) 250 subjects, age 30 - 94 years, from the Finnish Health 2000 Health Examination Survey and 2) 75 dizygotic (DZ) twin and 46 monozygotic (MZ) twin pairs, of which 20 MZ pairs are discordant for BMI (BMI-difference >3 kg/m^2 units), age 22 - 33 years, from the FinnTwin12 and FinnTwin16 cohorts.

In Health 2000 subjects, Angptl4 levels were negatively correlated with BMI ($r = -0.172$; $p = 0.008$) after adjustments. Within the age range 30 - 45 years ANGPTL4 levels were significantly decreased ($p = 0.03$) in overweight subjects (8.8 ± 1.3 ng/ml, $n = 23$) compared to normal weight subjects (17.5 ± 2.9 ng/ml, $n = 41$). In MZ pairs discordant for BMI, Angptl4 levels were significantly decreased ($p = 0.04$) in obese twins (14.8 ± 3.48 ng/ml) compared to their non-obese co-twins (43.3 ± 19.7 ng/ml, $n = 41$). Furthermore, in all MZ twins, intra-pair differences of Angptl4 serum levels were inversely correlated with intra-pair differences in liver fat ($r = -0.66$, $p = 0.001$), intra-abdominal fat ($r = -0.48$, $p = 0.036$) and serum triglycerides ($r = -0.34$, $p = 0.023$).

Our results suggest a role for Angptl4 in acquired obesity and in the crosstalk between liver and adipose tissue.

Poster Session

LESS ATHEROGENIC PROFILE OF TRIGLYCERIDES-RICH LIPOPROTEINS AFTER EZETIMIBE IN TYPE 2 DIABETIC PATIENTS

Lutgarda Bozzetto, G. Annuzzi, G. Della Corte, L. Patti, P. Cipriano, A. Strazzullo, L. Di Capua, G. Riccardi, A.A. Rivellese

Department of Clinical and Experimental Medicine, Federico II, Naples, Italy

Background and aims: Ezetimibe reduces LDL cholesterol by inhibiting cholesterol absorption, and may affect postprandial triglyceride-rich lipoproteins, i.e. the high atherogenic particles that are increased in type 2 diabetes. We evaluated how ezetimibe influences chylomicrons and VLDL particles at fasting and after a standard meal.

Patients and methods: Fifteen subjects with type 2 diabetes and hypercholesterolemia followed a 6-week treatment with ezetimibe10mg+simvastatin20mg (EZE+S) and placebo+simvastatin20mg (P+S)according to a randomized cross-over design . Trglycerides, cholesterol and apo B100 concentrations in total plasma and lipoprotein fractions (separated by discontinuous density gradient ultracentrifugation) were determined before and after a high-fat test-meal (6 hours).

Results: At fasting, EZE+S, compared with P+S, significantly decreased cholesterol in chylomicrons (0.83±1.41 *vs.* 0.19±0.12 mg/dl, M±SD, $p< 0.05$), IDL (6.5±1.9 *vs.* 4.7±2.0 mg/dl, $p< 0.05$), and LDL (76.9±21.2 *vs.* 56.5±13.6 mg/dl, $p< 0.001$), and triglyceride in chylomicrons (10.9±17.8 *vs*. 3.6±2.6 mg/dl, $p< 0.05$).

Postprandial total AUCs were lower after EZE+S than after P+S for cholesterol (chylomicron 4.4±2.7 *vs.* 8.3±8.7, IDL 22±8 *vs.* 31±9, LDL 334±79 *vs.* 452±116 mg/dlx6h; $p< 0.005$ for all).

Fasting and postprandial plasma apo B100 were significantly lower after EZE+S.

Conclusions: A 6-week treatment with ezetimibe and simvastatin, compared to simvastatin alone, in type 2 diabetic patients significantly reduced cholesterol and triglyceride content of chylomicrons and cholesterol content of remnant lipoproteins, beside its effects on LDL cholesterol. These effects were obtained both postprandially and at fasting, reflecting a prolonged action on intestinally derived lipoproteins, that induces a less atherogenic lipoprotein profile with ezetimibe.

Poster Session

DIETARY FATTY ACIDS INFLUENCE ENDOTHELIAL FUNCTION DURING ACUTE NEFA ELEVATION

Abby Thompson, K. Newens, K. Jackson, C. Williams

Food and Nutritional Sciences, University of Reading, Reading, UK

Increased NEFA affects insulin metabolism, and insulin mediates endothelial function through the activation of endothelial nitric oxide synthase. Elevated circulating NEFA has been shown to impair endothelial function, but very little is known about the impact of NEFA enriched in different dietary fatty acids.

Fifty healthy subjects were given drinks either rich in SFA (0.42g palm stearin/kg bodyweight) or SFA plus n-3 PUFA (0.36g palm stearin + 0.06g DHA-rich fish oil/kg bodyweight) on separate occasions. The drinks were given as a bolus (≈33% of total fat) followed by smaller amounts (≈11%) every 30 min during the 4 h study day. After 2 h, an intravenous infusion of heparin was used to increase circulating NEFA levels. Endothelial function was measured using FMD at baseline and 3.5 h.

The changes in NEFA levels during the heparin infusion were similar on both study days. During consumption of SFA, there was a reduction in the FMD response (impaired endothelial function), while there was an increase in FMD (enhanced endothelial function) with SFA plus n-3 PUFA. This observation was supported by a larger reduction in circulating nitrate levels during the SFA study day, suggesting a reduction in the amount of bioavailable nitric oxide.

Our study has shown the effect of raised NEFA on endothelial function may be dependent on the fatty acid composition of NEFA rather than simply the total NEFA concentration, and moderate substitution of n-3 PUFA for SFA may have a positive effect on endothelial function even at high NEFA levels.

Poster Session

HIV INFECTION INDUCES MAJOR CHANGES IN HDL PARTICLE SIZE DISTRIBUTION AND COMPOSITION: THE EFFECT OF ANTIRETROVIRAL TREATMENT AND DISEASE SEVERITY

Gerard Aragones[1], R. Beltrán-Debón[1], A. Rull[1], L. Fernández-Sender[2], J. Camps[1], J. Joven[1], C. Alonso-Villaverde[1,2]

[1]Centre de Recerca Biomèdica, Institut Investigació Sanitària Pere Virgili, Hospital Universitari Sant Joan, Universitat Rovira i Virgili, [2]Medicina Interna, Hospital Universitari Sant Joan de Reus, Rovira i Virgili, Reus, Spain

Objective: To evaluate whether HIV infection and antiretroviral treatment promote changes in cholesterol distribution among subpopulations of HDL particles with defined sizes.

Methods: HDL particles were isolated from 66 HIV-infected patients and fractionated by gel permeation chromatography to obtain five HDL particle subpopulations. Thirty-six of the participants were not receiving any antiretroviral therapy, while thirty patients were undergoing treatment with an NNRTI-based antiretroviral therapy. Uninfected control individuals were also included.

Results: HIV infection induced profound changes in HDL particle that correlated with the severity of the disease. The distribution of cholesterol across HDL particle sizes was also affected by HIV infection itself. Treatment (i.e., antiretroviral therapy) reduced these alterations; only minor changes in small and very small HDL particles were observed in treated patients ($P = 0.01$). Untreated patients with low CD4+T cell counts had less cholesterol in medium ($P = 0.006$), small ($P = 0.04$) and very small ($P = 0.03$) HDL particles. NNRTI-treated patients with high CD4+T cell counts had less cholesterol in the largest HDL particles ($P = 0.04$), with overall particle distributions resembling those observed in uninfected participants.

Conclusions: HIV infection promotes major changes in HDL particle. Current treatments may partially attenuate this effect.

Poster Session

SMALL-DENSE LDL IS A BETTER DETERMINANT OF PLASMA GLYCATED APOLIPOPROTEIN B THAN GLYCAEMIA IN METABOLIC SYNDROME AND TYPE2 DIABETIC PATIENTS

Nahla Younis[1,2], R. Sharma[1], H. Soran[1], V. Charlton-Menys[1], M. Elseweidy[2], P. Durrington[1]

[1]The University of Manchester, Manchester, UK, [2]Zagazig University, Faculty of Pharmacy, Biochemistry Department, Zagazig, Egypt

Glycation of LDL may be at least as important as oxidation in atherogenesis. Small-dense LDL (SD-LDL) has been particularly implicated in atherosclerosis in diabetes and metabolic syndrome (MS). We previously reported that in non-diabetic people SD-LDL is preferentially glycated. The distribution of glycated apolipoprotein B (glyc-apoB) in lipoproteins in MS and in type 2 diabetes has not previously been studied. In this study plasma apoB and glyc-apoB were determined by immunoassay in different apoB-containing lipoproteins including buoyant LDL (D=1.019-1.044g/ml) and SD-LDL (D=1.044-1.063g/ml) in 18 people with MS and 48 patients with type 2 diabetes [12 statin-untreated (DM) and 36 statin-treated (DM+S)]. Plasma glyc-apoB was 5.6±0.9 (mean±SEM), 3.5±0.5 and 4.0±0.2mg/dl in DM, DM+S and MS, respectively. The proportion of glyc-apoB in SD-LDL was greater than other lipoproteins in all 3 groups. SD-LDL contributed most to plasma glyc-apoB in DM, because SD-LDL-apoB was higher in DM than in MS and DM+S ($P< 0.001$). Plasma glyc-apoB correlated with SD-LDL-apoB in diabetes (r=0.74, $P< 0.0001$) and in MS (r=0.53, $P< 0.001$), but not with HbA_{1c}. In conclusion, SD-LDL is more likely to be glycated than buoyant LDL in both type 2 diabetes and MS. Its concentration is a stronger determinant of plasma glyc-apoB than glycaemia and statin-induced changes in its level may be important in decreasing apoB glycation in diabetes. These findings may explain the relatively moderate effect of improving glycaemic control as opposed to statin treatment in reducing atherosclerosis risk and the increased risk in MS even before the onset of diabetes.

Poster Session

ABNORMAL LPL RELEASE LEADS TO CHYLOMICRONEMIA IN THE PRESENCE OF GPIHBP1 DEFECTS

Benoit J Arsenault[1], A.P. Beigneux[2], F. Peelman[3], R. Franssen[1], J. Hertecant[4], S.G. Young[2], G.M. Dallinga-Thie[1]

[1]Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands, [2]Department of Medecine, David Geffen School of Medicine, UCLA, San Francisco, CA, USA, [3]Department of Biochemistry, Ghent University, Ghent, Belgium, [4]Tawam Hospital, Al Ain, United Arab Emirates

Introduction: In mice, glycophosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) and lipoprotein lipase (LPL) are critical determinants of triglyceride lipolysis. GPIHBP1 is expressed on the luminal site of microvascular endothelium cells in tissues that rely on fatty acid delivery. However, the relevance of GPIHBP1 for human biology remains to be established.

Results: Here we report a second homozygous missense mutation in GPIHBP1 identified in a patient with severe chylomicronemia. The mutation (C65Y) is a substitution of a tyrosine for a highly conserved cysteine located in the Ly-6 domain of GPIHBP1. A structural model of GPIHBP1 suggests that the C65Y mutation may affect the basic structure of the protein. Extensive cell culture experiments reveal that GPIHBP1-C65Y is displayed on the cell surface but has lost the capacity to bind LPL. One injection of heparin was ineffective to release LPL into the plasma compartment in both probands (GPIHBP1-Q115P and GPIHBP1-C65Y), indicating that in the absence of functional GPIHBP1, LPL cannot bind to the endothelial cell surface. A 6-hour heparin infusion in the proband with the GPIHBP1-Q115P mutation, however, decreased plasma TG concentrations from 20 mmol/L to 6 mmol/l.

Conclusions: Mutations in *GPIHBP1* leading to a defective protein are indeed functionally relevant in men and together with the observation that human GPIHBP1 and LPL share the same tissue distribution, these data decisively demonstrate that GPIHBP1 is instrumental for lipolysis in humans.

Poster Session

FEATURES OF THE INTIMAL-MEDIAL THICKNESS AND ENDOTELIAL FUNCTION IN PATIENTS WITH CORONARY ARTERY DISEASE & THE METABOLIC SYNDROME

Kateryna Yanovska

Department of Dislipidemiy, Institute of Cardiology, Kyiv, Ukraine

Aims: To learn the features of endotelial function and intimal-medial thickness (IMT) in patients with coronary artery disease (CAD) & the metabolic syndrome (MS) depending on the presence of the type II diabetes.

Methods: 70 patients with CAD, MS, which made two clinical groups: 1-st group from CAD, MS and DT II; 2-nd group from CAD, MS without type II diabetes were examined. All patient were determinated: anthropometric data, lipides profile, measuring of IMT general carotid artery, endotelial function of brachial artery.

Results: There were discovered that in the 1-st group the characterizes of IMT were higher than in the 2-nd group. The most closely positive correlation between descriptions of IMT and levels of cholesterol (r=0,68, p< 0,001), LDL-cholesterol (r=0,69, p< 0,001) in the 1-st group was found, also in this group there was negative correlation between the data of lipid metabolism and endotelial function (cholesterol r=-0,53, p< 0,001, LDL-cholesterol r=-0,55, p< 0,001). Although in the 2-nd group the faint direct correlation between the levels of cholesterol (r=-0,14, p< 0,001), LDL-cholesterol (r=0,17, p< 0,001) and IMT. In this group also found the closely negative correlation between the endotelial function and levels of cholesterol (r=-0,77, p< 0,001), LDL-cholesterol (r=-0,74, p< 0,001).

Conclusions: The type II diabetes in patients with CAD & MS associate with the structurally-functional changes of arterial vessels (increase IMT). Predominate functional changes of endotelial function have negative correlation with the data of lipid profile in patients with CAD & MS without impaired of carbohydrate metabolism.

Poster Session

INTERACTIONS OF APOLIPOPROTEIN A-I MUTANTS WITH ENDOTHELIAL CELLS

Pascale Ohnsorg[1], L. Rohrer[1], A. Chroni[2], V. Zannis[3], A. von Eckardstein[1]

[1]Institute of Clinical Chemistry, University Hospital, Zurich, Switzerland, [2]National Centre of Scientific Research 'Demokritos', Institute of Biology, Athens, Greece, [3]Department of Medicine and Biochemistry, Boston University Medical Center, Boston, MA, USA

High density lipoproteins (HDL) and apolipoprotein A-I (apoA-I), exert anti-atherogenic properties within the arterial wall. We reported that aortic endothelial cells (ECs) cultivated on inserts bind, internalize, and translocate apoA-I and HDL from the apical to the basolateral compartment. The specific transport involves either ABCA1 (apoA-I) or ABCG1 and SR-BI (HDL). To further investigate the transendothelial apoA-I transport we compared the endothelial cell interactions of wild type (WT) apoA-I and apoA-I mutants. The C-terminal deletion mutant apoA-I(Δ185-243), which cannot induce ABCA1- dependent lipid efflux from macrophages, showed 50% reduced total binding and no specific binding. The specific transport of this mutant was reduced even by 90%. But the N-terminal deletion mutant apoA-I(Δ1-59) and the double deletion mutant apoA-I(Δ1-59,Δ185-243), which both induce normal ABCA1 mediated lipid efflux in macrophages, showed 100 fold increased total binding which was not competed by WT apoA-I. Total transport of the two mutants was normal but could not be competed with WT apoA-I.

These data indicate the importance of WT apoA-I structure for specific binding and transport. We hypothesize that the C-terminus is important for the specific interactions of apoA-I with ABCA1 and specific transendothelial transport whereas the amphipathic midregional alpha helices of apoA-I provide the basis for unspecific interactions with the lipid-bilayer of the plasma membrane which in the absence of the C-terminus are increased. We also show that oligomerized apoA-I is normally bound and transported by cultured aortic endothelial cells.

Poster Session

USEFULNESS OF INTIMA MEDIA THICKNESS, APOB AND NON-HDL-C IN PRIMARY DISLIPIDEMIC CHILDREN

Viviana Baracco, F. Abello, O. Guardamagna

Department of Pediatrics, University of Turin, Turin, Italy

Objective: Aim of the study was to check the relationship between carotid IMT (cIMT) and aorta IMT (aIMT) with biochemical risk markers of atherosclerosis in a children cohort.

Methods: 329 healthy and dyslipidemic children/adolescents (age 10.10±3.40) underwent B-mode ultrasound. Among dyslipidemic children 88 resulted FH, 74 FCHL, 56 dominant Hypercholesterolemia, 43 Hyperlipoprotein(a), 10 FHTG and 1 sitosterolemia. 57 were unaffected control subjects. cIMT and aIMT were assessed by using B-mode ultrasound.

Results: In all subjects IMT measurement increased with age independently from gender. Both cIMT and aIMT correlated respectively with LDL-C (ρ=0.129, p=0.020; ρ=0.124, p=0.041), ApoB (ρ=0.150, p=0.007; ρ=0.158, p=0.01), non-HDL-C (ρ=0.128, p=0.021; ρ=0.138, p=0.023) and ApoB/$ApoA_1$ (ρ=0.185, p=0.001; ρ=0.141, p=0.022). A negative correlation was found between cIMT and $ApoA_1$ (ρ=-0.179, p=0.01). Furthermore aIMT resulted significantly related to CT levels (ρ=0.128, p=0.034).

Conclusion: The present study shows, for the first time in children, a correlation between cIMT and aIMT with LDL-C, ApoB, ApoB/$apoA_1$, non-HDL-C, as well as aIMT with CT. ApoB and $ApoA_1$, as well as non-HDL-C, are accurate summary of CVD risk factors and are considered new good marker risk as regards to the conventional ones. It should be underlined that non-HDL-C can be routinely assessed in the clinical practice because it results an easy and cheap measure already useful in hypertrigliceridemic patients. This study support the effectiveness of ApoB and non-HDL-C values, as well as cIMT and aIMT measurements, as preclinical markers of atherosclerosis in childhood.

Poster Session

INFLUENCE OF BODY FAT DISTRIBUTION, DIET AND EXERCISE ON THE FORMATION OF NITROTYROSINE IN THE HEART OF RATS

Cleverson Rodrigues Fernandes, K.M.A. Mata, F.M. Mauad, F.T.T. Frajacomo, A.P. Farnesi, V.K. Cardoso, T. Marini, J.E. Junior, S.A. Uyemura, S.G. Ramos, S.B. Garcia

University of São Paulo, Ribeirão Preto, Brazil

Introduction: High-fat diet (HFD) and exercise alter the body composition, nitrotyrosine formation, glycemia and cholesterolemia, wich can be dangerous for the cardiovascular system and endothelial function. However, caloric restriction (CR) reduces the production of reactive oxygen species and metabolic rate.

Methodology: Ninety two-month-old male Wistar rats were randomly assigned in nine groups: (G1) standard diet control, (G2) CR diet control, (G3) HFD control, (G4) standard diet 20-minutes training, (G5) CR diet 20-minutes training, (G6) HFD 20-minutes training, (G7) standard diet 90-minutes training, (G8) CR diet 90-minutes training, (G9) HFD 90-minutes training. Rats swam 5 days a week, for 8 weeks.

Results: Diets were effective in body weight variation (G1,G2, G3), and did not influence in the heart's weight in all groups. Glycemia and cholesterol increased just in G3. The 90 minutes of physical exercise caused left ventricular hypertrophy, with exception of the CR groups. The computed tomography showed that abdominal, visceral and cardiac areas were changed with diets and exercise. Ventricular fat area was not modified; levels of nitrotyrosine were elevated only in groups G6 and G9.

Conclusion: The distribution of body fat, does not influence in the quantity of cardiac fat muscle ventricular. HFD associated to exercise, powers the left ventricular hypertrophy, and increases the nitrotyrosine formation, which was not decrease by exercise and CR.

Poster Session

EFFECT OF APOCIII SSTI POLYMORPHISM IN POSTPRANDIAL RESPONSE TO A FAT OVERLOAD IN METABOLIC SYNDROME PATIENTS

Mercedes Clemente-Postigo[1], M. Queipo-Ortuño[2], F.J. Tinahones[2], F. Cardona[1]

[1]Endocrinologia y Nutrición, Hospital Clínico Universitario Virgen de la Victoria (IMABIS), [2]Endocrinologia y Nutrición, Hospital Clínico Universitario Virgen de la Victoria (IMABIS).CIBEROBN, Málaga, Spain

Objectives: Apolipoprotein CIII is a major component of VLDLs and chylomicrons and minor component of HDLs. It has been shown its implication in TRLs catabolism and the SstI polymorphism has been associated with hipertriglyceridemia in patients and healthy subjects. We rise that it can exit an association between the minor S2 allele and the postprandial response of triglyceride in metabolic síndrome (MS) patients.

Methods: We selected 72 patients with MS according ATPIII criteria and 21 healthy subjects and underwent 60g fat overload with a commercial preparation, it was measured lipid profile and the APOCIII SstI genotype by RFLPs (Restriction Fragments Length Polymorphism).

Results: We found significant differences in fasting triglyceride (P=0,048), 3h triglyceride (P=0,002) and 4h triglyceride (P=0,002) levels in patients with S2S2 genotype respect S1S1 and S1S2 patients. Differences in postprandial triglyceride levels were influenced by genotype and fasting triglyceride levels, but not by gender or age. The triglyceride increase respect baseline was also higher in S2S2 homozygous patients than in WT patients at 4h (P= 0,001) pointing out that all S2S2 MS patients had a triglyceride increase higher than 150mg/dL at 4h (P=0,028). In addition, S2S2 patients had incremented chylomicron-TG (P=0,006) respect the other genotypes. Controls didn't showed significant variations in triglycerides levels respect genotype and other lipids parameters were not affected by the APOC3 genotype.

Conclusion: Homozygosis for the minor SstI polymorphism of APOC3 gene was associated with a higher postprandial plasma triglyceride levels as well as elevated postprandial chylomicron-TG levels in patients but not in controls.

Poster Session

COMMON GENETIC VARIATION IN *APOA5*, LEVELS OF TRIGLYCERIDES AND RISK OF MYOCARDIAL INFARCTION IN THE GENERAL POPULATION

Anders Berg Jørgensen[1], R. Frikke-Schmidt[1], P. Grande[2], B.G. Nordestgaard[3], A. Tybjærg-Hansen[1]

[1]Dept. Clinical Biochemistry, [2]Dept. Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, [3]Dept. Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark

Introduction: Apolipoprotein A-V is important for the metabolism of triglyceride-rich lipoproteins, and genetic variation in *apolipoprotein A5* (*APOA5*) is known to influence levels of triglycerides. It is however unknown at present whether genetic variation in *APOA5* predisposes to myocardial infarction (MI). We tested whether single nucleotide polymorphisms (SNPs) in *APOA5* affect triglyceride levels and risk of MI in the general population.

Methods: We screened the *APOA5* gene in individuals with the lowest 1% (n=95) and highest 2% (n=190) triglyceride levels for age and gender among participants from the Copenhagen City Heart Study (CCHS, n=10,300). Subsequently, we genotyped those four regulatory or protein coding *APOA5* SNPs that differed in frequency between the extreme groups of triglyceride levels, in the entire CCHS, and in the Copenhagen Ischemic Heart Disease Study (CIHDS, n=2,825 cases).

Results: -1131T>C, -3A>G, S19W, and *31C>T differed in frequency between the extreme groups of triglyceride levels. In the entire CCHS, levels of triglycerides increased as a function of -1131T>C, -3A>G and S19W genotype from noncarriers to heterozygotes to homozygotes, and decreased as a function of *31C>T genotype from noncarriers to heterozygotes to homozygotes (all P for trends: < 0.001). Hazard ratios for MI increased up to 1.7 in the CCHS (P for trend: < 0.05) and up to 2.1 in the CIHDS (P for trend: < 0.001) with increasing number of triglyceride increasing alleles.

Conclusion: Common genetic variation in *APOA5* is associated with levels of triglycerides and a corresponding increase in risk of MI in the general population.

Poster Session

FATTY ACIDS DERIVATIVES BY LIPID PEROXIDATION ARE SIGNIFICANTLY INCREASED IN PATIENTS WITH HIGH APOLIPOPROTEIN B-48 LEVELS, INDEPENDENT OF TRIGLYCERIDE CONCENTRATIONS

Daisaku Masuda[1], H. Nakaoka[1], R. Kawase[1], M. Inagaki[1], M. Yuasa-Kawase[1], K. Nakatani[1], K. Tsubakio-Yamamoto[1], J.C. Sandocal[1], T. Ohama[1], Y. Nakagawa-Toyama[1,2], M. Nishida[1,2], M. Ishigami[1,3], N. Ishida[4], M. Shichiri[4], Y. Yoshida[4], I. Komuro[1], S. Yamashita[1]

[1]Department of Cardiovascular Disease, Osaka University Graduate School of Medicine, Suita, [2]Health Care Center, Osaka University, Toyonaka, [3]Division of Health Sciences, Osaka University Graduate School of Medicine, Suita, [4]Health Technology Research Center, National Institute of Advanced Industrial Science and Technology, Ikeda, Japan

Background and aim: The increase in reactive oxygen species (ROS) is closely related to the morbidity of atherosclerotic cardiovascular diseases. Recently, many reports suggest that chylomicron remnants (CM-R), which are the hydrolyzed products of intestine-derived chylomicrons (CM) and significantly increases in the postprandial state, play a key role in the development of atheromatous plaques and increases risk status of cardiovascular diseases. We investigated the correlation between CM-R and ROS.

Methods: Subjects were collected who underwent annual health check-up at Health Care Center of Osaka University (n=99, m:f=70:29, 39±10 years old of age). Fasting levels of LDL-cholesterol, HDL-cholesterol, TG, apolipoproteinB-48, remnant lipoprotein cholesterol (RemL-C), linoleic acids were measured and lipid peroxidation derivatives of free fatty acids, hydroxyoctadecadienoic acids (HODE) and those of arachidonic acids, hydroxyeicosatetraenoic acids (HETE) were analyzed by GC/MS.

Results: There was no significant difference in ROS parameters, HODE, HETE and HODE/linoleic acid (H/L) ratio, when subjects were divided by LDL-C (140mg/dl), HDL-C (50mg/dl), RemL-C (7.5mg/dl) and TG(150mg/dl) levels. However, these levels were significantly higher in subjects with high apoB-48 levels (>2.8mg/dl) than in those with low apoB-48 levels. Moreover, when subjects were divided into two groups by TG level (150 mg/dl), ROS parameters were significantly higher in high apoB-48 subjects (>5.9 mg/dl in high TG group, >2.7 mg/dl in low TG group) than in low apoB-48 subjects.

Conclusion: ROS parameters (HODE, HETE and H/L ratio) were significantly higher in subjects with high apoB-48 levels independent of TG levels, suggesting that ROS was highly stimulated by CM-R accumulation.

Poster Session

COMMON GENETIC VARIATION IN *APOA1* CONTRIBUTES TO ELEVATED HDL CHOLESTEROL IN THE GENERAL POPULATION

Christiane L. Haase[1], R. Frikke-Schmidt[1], B.G. Nordestgaard[2,3], G. Jensen[3], A. Tybjærg-Hansen[1,3]

[1]Dept. Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet, Copenhagen, [2]Dept. Clinical Biochemistry, Herlev Hospital, Herlev, [3]The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen, Denmark

Introduction: Epidemiologically, high-density lipoprotein (HDL) cholesterol levels are inversely related to risk of ischemic heart disease. We tested whether common genetic variation in apolipoprotein A-I (apoA-I), the major protein constituent of the HDL particle, contributes to HDL cholesterol and apoA-I levels in the general population.

Methods: We resequenced the regulatory and coding regions of *APOA1* in 190 individuals from The Copenhagen City Heart Study (CCHS) with the lowest 1% (*n*=95) and highest 1% (*n*=95) apoA-I levels. Identified common genetic variants were genotyped in CCHS(*n*=10,355) and the effect on lipid and lipoprotein levels determined.

Results: Resequencing *APOA1* identified six common genetic variants. Three variants (g.-560A>C, g-151C>T, *181A>G) were in strong pairwise linkage disequilibrium (all r^2>0.85), and the frequency of the haplotype including the minor alleles (CTG) differed between the high and low apoA-I group (6% versus 1%, P=0.002). Two variants, g.-560A>C (tagging the CTG haplotype above) and g.-310G>A, were genotyped in CCHS (allele frequencies, respectively, 0.04 and 0.19). In agreement with findings above, HDL cholesterol levels were increased by 0.07 mmol/L in g.-560A>C carriers (P=0.001); the corresponding increase in apoA-I levels were 5 mg/dL (P< 0.001). In g.-310G>A homozygotes, the plasma levels of HDL cholesterol and apoA-I were increased, respectively, by 0.11mmol/L (P< 0.001) and 8mg/dL (P< 0.001). Combining the two common genetic variants showed an additive effect on plasma levels of HDL cholesterol (0.12 mmol/L, P=0.007) and apoA-I (10 mg/dL, P< 0.001) in compound heterozygotes.

Conclusion: Common genetic variation in *APOA1* contributes to HDL cholesterol levels in the general population.

Poster Session

THE VALUE OF THE APOB/APOA1 RATIO FOR THE DIAGNOSIS OF INSULIN RESISTANCE

Laura Lopez-Rios[1,2], J.C. Wiebe[1], A.M. Wägner[1,2], Y. Brito-Casillas[1], M. Lorenzo-Media[3], J.A. Aguilar-Doreste[3], N. Pérez-Martín[1], R. Chirino[4], J. Nóvoa[1,2], M. Boronat[1,2]

[1]Section of Endocrinology and Nutrition, Hospital Universitario Insular, Las Palmas de GC, [2]Department of Medical and Surgical Sciences, Las Palmas University, La Palmas de GC, [3]Service of Clinical Biochemistry, Hospital Dr. Negrín, Las Palmas de GC, [4]Department of Biochemistry, Molecular Biology, Fisiology, Genetics and Immunology, Las Palmas University, Las Palmasde GC, Spain

Objective: To determine the contribution of the apoB/apoA1 ratio to the identification of subjects with insulin resistance.

Methods: We selected 837 non-diabetic individuals (age ≥30 years) from a population-based study performed in the island of Gran Canaria (Spain). Insulin resistance was defined as a HOMA value higher than the 75th percentile.

Results: In a logistic regression analysis with stepwise selection of variables, the apoB/apoA1 ratio was independently associated with insulin resistance (OR: 4.195, 95% CI: 1.320-13.331; p=0.015), along with age, waist perimeter, triglycerides, fasting glucose, systolic pressure and current use of hypolipidemic and antihypertensive drugs; in contrast, HDL-cholesterol levels and diastolic blood pressure were excluded from the model.Using ROC curves, the best cut-off points of apoB/apoA1 ratio to discriminate between men and women with or without insulin resistance were 0.68 for men and 0.61 for women. According to these cut-off values, the apoB/apoA1 ratio was also independently associated with insulin resistance in a logistic regression analysis that included age, sex and the metabolic syndrome (as defined by the NCEP-AHA or the IDF), introduced either as a single variable (OR: 1.840; 95%CI: 1.250-2.709; p=0.011) or through the inclusion of its five individual components (OR: 1.729; 95%CI: 1.134-2.635; p= 0.002).

Conclusion: The apoB/apoA1 ratio is a strong and independent predictor of insulin resistance. Values of apoB/apoA1 ratio higher than 0.68 for men and 0.61 for women are associated with insulin resistance, independently of the presence of the metabolic syndrome or its individual components.

Poster Session

APOB48/TG RATIO IS A NOVEL AND USEFUL MARKER FOR THE DETECTION OF TYPE III HYPERLIPIDEMIA AFTER ANTIHYPERLIPIDEMIC INTERVENTIONS

Hajime Nakaoka[1], M. Yuasa-Kawase[1], D. Masuda[1], R. Kawase[1], K. Nakatani[1], M. Inagaki[1], J.C. Sandoval[1], K. Tsubakio-Yamamoto[1], T. Ohama[1], Y. Nakagawa-Toyama[1,2], M. Nishida[1,2], M. Ishigami[1,3], I. Komuro[1], S. Yamashita[1]

[1]Department of Cardiovascular Disease, Osaka University Graduate School of Medicine, Suita, [2]Health Care Center, Osaka University, Toyonaka, [3]Division of Health Sciences, Osaka University Graduate School of Medicine, Suita, Japan

Background: Type III hyperlipidemia (HL) is caused by the genetic polymorphisms of apolipoprotein(apo)E (apoE2/2) and diagnosed by inconvenient methods of phenotyping apoE using Western blotting or isoelectric focusing. Formerly we found the simple marker for their detection, high apoB-48/triglycerides (TG) ratio among other types of HL (*J Lipid Res,* 2003;44:1256). However, HL patients are promptly treated with antihyperlipidemic drugs without diagnosing its etiology, resulting in some modifications of their lipid profiles.

Aim: To investigate whether apoB-48/TG ratio may also be a simple and useful marker for the detection of type III HL after antihyperlipidemic interventions.

Patients and methods: Consecutive outpatients of Cardiovascular Division in Osaka University Hospital were collected for this study with informed consent (25 normolipidemic subjects and 127 treated HL patients, TypeI, n=3; IIa, n=48; IIb, n=48; III, n=7; IV, N=18; V, n=3, respectively). Fasting LDL-C, HDL-C, TG and apoB-48 levels were measured and their apoB-48/TG ratios were calculated.

Results: ApoB-48 level was significantly higher in type I, III and V HL patients among other types of HL. However, the ApoB-48/TG ratio was significantly higher only in type III HL, but not in type I or type V HL. These results suggest that CM or CM remnants were accumulated in type III HL patients even though antihyperlipidemic drugs had already been administrated.

Conclusion: The ApoB-48/TG ratio is significantly high in type III HL patients irrespective of antihyperlipidemic treatments and can be a novel useful marker for easy detection of type III HL without time-consuming electrophoretic analyses.

Poster Session

THE GENERATION OF NEW PRE-BETA HDL LIKE PARTICLES - A KEY MECHANISM OF THE ANTI-ATHEROGENIC ACTION OF LIPOSOMES?

Anna Wolska[1], M. Czyzewska[1], A. Szutowicz[1], A. Ćwiklińska[2], M. Wróblewska[1]

[1]Department of Laboratory Medicine, [2]Department of Clinical Chemistry, Medical University of Gdansk, Gdansk, Poland

Repeated phospholipids (PL) infusions reverse experimentally induces atherosclerosis in a variety of animal models. Our recent studies demonstrated that interaction between human HDLs and egg yolk lecithin (EYL) small unilamellar liposomes (SUV) generated a new pre-beta mobility lipoprotein fraction containing apo A-I and apo A-II. In this study we analyzed the redistribution of apo A-I, apo A-II, free cholesterol (FC) and PL between remodeled alpha-HDLs, a newly generated pre-beta mobility fraction and non-disrupted liposomes. Only a part of PL derived from liposomes was accessible for HDL particles. On the other hand pre-beta mobility fraction was a very efficient PL “scavenger”. At initial SUV-PL/HDL-PL ratios 1:1, 3:1 and 5:1 alpha-HDLs accepted approximately 17, 17 and 11% of liposomal PL, respectively. Simultaneously pre-beta mobility fraction gathered approximately 37, 38 and 36% of liposomal PL, respectively. At initial SUV-PL/HDL-PL ratios 1:1, 3:1 and 5:1 ratios approximately 77, 90, 85% of apo A-I dissociated from HDLs was found in the pre-beta mobility fraction, respectively. FC lost by HDLs was distributed evenly between pre-beta mobility fraction and liposomes at all three incubation ratios investigated. However, student´s t-test for paired samples showed that the mean value of FC fractional content in the pre-beta mobility fraction generated at SUV-PL/HDL-PL ratio 1:1 was significantly higher than in remodeled liposomes (p=0.05). This indicates that pre-beta mobility fraction accepted cholesterol more avidly than liposomes. We conclude, that the generation of new pre-beta HDL like particles may be a key mechanism of the anti-atherogenic properties of liposomes.

Poster Session

MOLECULAR DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA (FH) IN GREECE. KNOWN AND NOVEL MUTATIONS IN THE LOW DENSITY LIPOPROTEIN RECEPTOR (*LDLR*) GENE

V. Mollaki, **Pavlos Progias**, A. Skouma, E. Drogari

1st Department of Pediatrics, Choremio Research Laboratory, Unit of Metabolic Diseases, Aghia Sophia Children´s Hospital, University of Athens, Medical School, Athens, Greece

FH is a common autosomal dominant disorder that is characterized by elevated plasma concentrations of LDL and total cholesterol, causing premature atherosclerosis and coronary artery disease. The diagnosis of FH is indisputable only when the clinical parameters are supported by molecular data. In the Greek population, FH is caused by *LDLR* mutations. We aimed to characterize the *LDLR* in FH patients and to provide a genotype-phenotype correlation in the probands (children). The *LDLR* was screened by sequencing in 412 clinically diagnosed patients from 186 families. The 186 probands had 256 mg/dl mean total cholesterol and 174 mg/dl mean LDL, while 51.6% were positive for *LDLR* mutations. The commonest mutations, Gly528Asp, Ser265Arg, Val408Met, Cys6Trp and Cys152Arg accounted for >75% of all mutations. Thirteen additional mutations were identified, 5 of which were previously described in other populations but not in Greeks. Three novel variants, a duplication 13 at 661 leading to a frameshift, and the nonsynonymous changes Arg216Cys and Tyr354Ser at amino acids highly conserved across species were found. These variants segregated with high lipid levels and are probably disease-causing. No difference was found in lipid levels of children carrying the transport-defective or the recycling-defective mutations. The homogeneity of the Greek population makes the development of a quick screening method feasible. However it´s important for the diagnosis to define rare or novel mutations. Identification of *LDLR* mutations, especially in children, will certainly facilitate the prevention of premature atherosclerosis and coronary artery disease, and will be valuable for genetic counseling and prenatal screening.

Poster Session

INCREASED ATHEROSCLEROSIS MARKERS ARE ASSOCIATED WITH HIGHER ACTIVITIES OF PHOSPHOLIPID OR CHOLESTERYL ESTER TRANSFER PROTEINS IN ADULTS WITH HIGH HDL-CHOLESTEROL

I.F. Espirito Santo[1], F.D. Santiago[1], R.T. Nakamura[2], M. Gidlund[3], P.M. Cazita[3], **Eliana Cotta de Faria**[1]

[1]Department of Clinical Patology, University of Campinas, [2]Diagnostic Image Laboratory, [3]Imunology and Endocrinology Departments, University of São Paulo, Campinas, Brazil

Aim: Phospholipid Transfer Protein (PLTP) has the ability to initiate inflammation and to increase apolipoprotein B-containing lipoproteins along with Cholesteryl Ester Transfer Protein (CETP). We aimed at determining the association of hsCRP, TNF-alpha and lipoprotein (a) with these lipid transfer proteins and early carotid atherosclerosis in healthy individuals presenting high HDL-Cholesterol (C).

Methods: Healthy individuals, 70%F and 30%M, with HDL-C ≥ 68 mg/dL according to HDL-C 90th percentile value, (n= 131, aged 52 ± 13y) and controls (n=131, HDL-C ≥40 and ≤ 67mg/dL) were selected and classified by tertiles of hsCRP, TNF-alpha and lipoprotein (a). PLTP, CETP, HL and LPL activities were determined. Carotid ultrasound was performed.

Results: In the group with high HDL-C the highest hsCRP tertile (≥0.3mg/L, n=45), higher PLTP activity was observed (23 ± 10% of transfer, + 26%) but no differences in CETP, HL and LPL activities were found, as well as in carotid IMT. In the highest Lp(a) tertile (≥42mg/dL, n=41), CETP activity was increased (10 ± 8% of transfer, + 23%). No associations were found for TNF-alpha.

Conclusions: We show that plasma PLTP associates positively with hsCRP in adults without clinical inflammation and that CETP is positively related to Lp(a).

The increases in activities of components of the reverse cholesterol transport, without repercussions on early atherosclerosis, could be due in part to high HDL-C levels.

Poster Session

ATHEROSCLEROTIC AND METABOLIC REPERCUSSIONS OF INCREASED PLASMA LEVELS OF OXIDIZED LDL AND ANTIBODIES AGAINST OXIDIZED LDL IN ASYMPTOMATIC ADULTS

F.D. Santiago[1], P. Moriel[1], R.O. Correa[1], R.T. Nakamura[2], M. Gidlund[3], R. Schreiber[1], S. Barros-Mazon[1], **Eliana Cotta de Faria**[1]

[1]Department of Clinical Pathology, [2]Radiology Department, University of Campinas, [3]Immunology Department, University of São Paulo, Campinas, Brazil

Aims: Oxidized LDL (oxLDL) is associated to atherosclerosis. Autoantibodies to epitopes of oxLDL (abLDLox) are markers of LDL oxidation. We aimed at correlating oxLDL and abLDLox with lipid transfer proteins and early carotid atherosclerosis in asymptomatic adults.

Methods: Healthy adults (n=169; 59% F and 41% M) were classified by tertiles of oxLDL and abLDLox. Lipids, lipoproteins, apolipoproteins AI and B100 were measured. Cholesteryl ester and phospholipid transfer proteins, hepatic lipase and lipoprotein lipase were determined by radiometric methods. OxLDL (Mercodia) and abLDLox were determined by ELISA. Carotid ultrasound was performed.

Results: In the highest oxLDL tertile (≥ 70 U/L, n=36) higher cholesterol (33%), LDL-C (40%), apoB100 (40%), reduced CETP (-29%), higher cIMT (33%) and a positive correlation between BMI and oxLDL ($p \leq 0.026$) were found. In the highest abLDLox tertile (≥0.35 OD n=34), reduced HL (-40%) and a borderline increase of cIMT (27%) were observed.

Conclusions: LDL oxidation and the immune response to it contributed to early carotid atherosclerosis by reducing reverse cholesterol transport in asymptomatic adults.

Poster Session

THE SUPPLEMENTATION OF COENZYME Q10 TO A MEDITERRANEAN DIET IMPROVES ANTIOXIDANT SYSTEMS AND REDUCES CELLULAR OXIDATION IN ELDERLY SUBJECTS

N. Delgado-Casado[1], E.M. Yubero-Serrano[1], P. Pérez-Martínez[1], I. Tasset-Cuevas[2], M. Santos-González[3], J. Delgado-Lista[1], **Antonio García-Ríos**[1], C. Marín[1], F. Fuentes[1], J.M. Villalba[3], I. Túnez[2], F. Pérez-Jiménez[1], J. López-Miranda[1]

[1]Reina Sofia University Hospital, Lipids and Atherosclerosis Research Unit, University of Cordoba, [2]Dept. of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cordoba, [3]Dept. of Cell Biology, University of Cordoba, Córdoba, Spain

Background: Postprandial oxidative stress is characterized by an increased susceptibility of the organism toward oxidative damage after consumption of a meal rich in lipids and/or carbohydrates. A diet with high content in antioxidants delays the atherogenic process by decreasing the degree of oxidative stress.

Objective: To determine whether the quality of dietary fat alters postprandial cellular oxidative stress and whether supplementation with CoQ lowers postprandial oxidative stress in an elderly population.

Design: Patients were randomly assigned to receive, in a crossover design, three isocaloric diets for periods of four-weeks each.

1. Mediterranean diet supplemented with CoQ (Med+CoQ diet).

2. Mediterranean diet (Med diet).

3. SFA-rich diet (SFA diet). After a 12-h fast, volunteers consumed a breakfast with a fat composition similar to that consumed in each of the diets. We determined levels of CoQ, lipoperoxide (LPO), oxidized LDL (oxLDL), F_2-isoprostanes, protein carbonyl (PC), nitric oxide (NO), endothelial function, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities.

Results: Med+CoQ breakfast induced higher postprandial levels of CoQ and improved capillary flow compared to subjects adhering to the Med and SFA meals ($p < 0.05$). Med+CoQ breakfast produced a postprandial higher decrease in LPO, F_2-isoprostanes, PC and antioxidant enzyme activities ($p < 0.05$), compared to the other two diets.

Conclusions: Mediterranean diet supplemented with CoQ improves postprandial oxidative stress in healthy elderly subjects. This model would be an appropriate therapy for processes that lead to a rise in oxidative stress, such as cardiovascular, neurodegenerative diseases and ageing.

Poster Session

EFFECTS OF IRON OVERLOAD-INDUCED OXIDATIVE STRESS

Henrike Arnold[1], K. Gerken[2], P. Nielsen[2], U. Wenzel[3], R. Böger[1], E. Schwedhelm[1]

[1]Institute of Experimental and Clinical Pharmacology and Toxicology, [2]Institute of Biochemistry and Molecular Biology II, [3]Department of Internal Medicine III, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: Haemochromatosis type 1 (HFE-related hereditary haemochromatosis, HH) leads to an iron overload e.g. in the liver. Iron overload induces reactive oxygen species (ROS) and leads to oxidative stress. We have recently demonstrated that patients with HH show elevated urinary excretion of 8-iso-$PGF_{2\alpha}$. Isoprostanes (IsoP) like 8-iso-$PGF_{2\alpha}$ are prostaglandin (PG)-like compounds, endogenous markers of oxidative stress, and independent risk markers of coronary heart disease (CHD).

Methods: We investigated the effects of iron overload-induced oxidative stress, using an established HFE animal model. Male Wistar Han rats were fed 3, 5, 5-trimethylhexanoyl ferrocene (TMH-ferrocene) or control diet (n=8/6) for 11 weeks. The urinary excretion of 8-iso-$PGF_{2\alpha}$ was measured by gas chromatography-mass spectrometry (GC-MS). Systolic blood pressure was measured by tail-cuff plethysmography in awake rats. In vitro vasoconstriction of isolated rat aorta was tested in organ bath experiments (n=5).

Results: The urinary excretion of 8-iso-$PGF_{2\alpha}$ was 4-fold higher in the iron-enriched diet group compared to control diet group (1600±640 pg/mg vs. 400±30 pg/mg creatinine; mean±SEM, $p< 0.05$). Systolic blood pressure was higher in the iron-enriched diet compared to control (117±9 mmHg vs. 106±6 mmHg; $p< 0.05$). In vitro 8-iso-$PGF_{2\alpha}$ constricted rat aorta by 100±5 % vs. 50±9 % by $PGF_{2\alpha}$ (3 µM; $p< 0.001$).

Conclusion: Experimental iron overload induces the formation of 8-iso-$PGF_{2\alpha}$ in rats and causes systolic hypertension. In vitro 8-iso-$PGF_{2\alpha}$ is a potent vasoconstrictor of rat aorta. We conclude that 8-iso-$PGF_{2\alpha}$ may be related to elevated blood pressure in experimental iron overload.

Poster Session

SPECIFIC OXIDATION OF APOLIPOPROTEIN-B-100 BY MYELOPEROXIDASE

Cédric Delporte[1], P. Van Antwerpen[1], K. Zouaoui Boudjeltia[2], C. Noyon[1], D. Calay[3], F. Reyé[1], A. Rousseau[2], M. Raes[3], M. Vanhaeverbeek[2], J. Ducobu[2], J. Nève[1]

[1]Laboratory of Pharmaceutical Chemistry, Université Libre de Bruxelles, Brussel, [2]Laboratory of Experimental Medicine, CHU Charleroi, Hopital A. Vésale, Université Libre de Bruxelles, Montigny-le-Tilleul, [3]Laboratory of Cellular Biology, University of Namur (FUNDP), Namur, Belgium

Atherosclerosis is an inflammatory disease characterized by the accumulation of lipids in the subendothelial space. Among the proatherogenic factors, the oxidative modifications of low-density-lipoproteins (LDLs) are frequently mentioned. Apolipoprotein-B-100 (apoB100) is the major protein of LDLs which stabilizes the particle, binds to the LDL receptor and plays a primordial role in lipoprotein metabolism. Although lots of experiments have been done on copper-oxidized LDLs, other more physiological pathways of oxidation are known and myeloperoxidase (MPO) could be one of it. Indeed, MPO is a peroxidase enzyme which catalyses the synthesis of hypochlorous acid (HClO), a powerful oxidant, in the presence of hydrogen peroxide (H_2O_2) and chloride.

The modifications of apoB100 under 3 different oxidative conditions were presently examined:

(i) oxidation of native LDLs with Cu (II),

(ii) with HClO (to mimic MPO process), and

(iii) with the MPO/H_2O_2/Cl^- system.

A total acid hydrolysis of the apoB100 from oxidized LDLs was carried out and amino acids and their oxidized products were analyzed by LC-MS/MS. No difference was observed for methionine oxidation under the 3 conditions whereas different lysine and tyrosine oxidation products were evidenced both in HClO and MPO/H_2O_2/Cl^- systems. As a matter of fact, HClO oxidation produced higher quantities of aminoadipic acid (lysine oxidative product) than of chloro-tyrosine whereas MPO oxidation produced similar amounts of aminoadipic acid and chloro-tyrosine. No difference was observed for tryptophan oxidation.

ApoB100 modifications are therefore different under the three experimental conditions and actually occur by MPO oxidation, which is physiologically relevant and specific.

Poster Session

A NOVEL HDL-ASSOCIATED PROTEIN, PROGRANULIN, IS INVOLVED IN LIPID METABOLISM AND MACROPHAGE RECRUITMENT INTO ADIPOSE TISSUES

Ryota Kawase[1], T. Ohama[1], A. Matsuyama[2], H. Okura[2], T. Matsuwaki[3], M. Yuasa-Kawase[1], K. Nakatani[1], M. Inagaki[1], J.C. Sandoval[1], K. Tsubakio-Yamamoto[1], D. Masuda[1], Y. Nakagawa-Toyama[4], M. Nishida[4], M. Ishigami[5], Y. Ohmoto[6], M. Nishihara[3], I. Komuro[1], S. Yamashita[1]

[1]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, [2]Institute of Biomedical Research and Innovation Laboratory, Foundation for Biomedical Research and Innovation, Kobe, [3]Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, Tokyo, [4]Health Care Center, [5]Division of Health Science, Osaka University Graduate School of Medicine, Suita, [6]Free Radical Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan

Progranulin (PGRN) is a multifunctional protein and recent reports that some patients with fronto-temporal dementia have mutations in PGRN gene have attracted much attention. PGRN is also known to be involved in tumorigenesis, systemic inflammation and wound healing. Recently, we have reported PGRN is expressed in and secreted from human monocyte- derived macrophages, and is bound to apolipoprotein (apo) A-I. In the current study, we investigated the possible association of PGRN with lipid metabolism and pathogenesis of metabolic syndrome. While plasma HDL-C levels were not different between the two groups, plasma TG levels of PGRN-KO mice, especially in VLDL fraction, were significantly higher than those of wild-type (WT) mice. The mRNA expressions of genes related to the synthesis of TG, such as SREBP1c and FAS, were significantly increased in the liver of PGRN-KO mice compared with those of WT mice. Plasma free fatty acid level of PGRN-KO mice was significantly higher and adiponectin level was significantly lower than that of WT mice. The mRNA expression of adiponectin in mesenteric fat of PGRN-KO mice on high fat diet was significantly decreased. The mRNA expressions of F4/80, CD68, MCP-1, IL-6 and TNF-alpha in mesenteric fat of PGRN-KO mice on high fat diet were significantly increased, suggesting that PGRN may be associated with the recruitment of macrophages to adipose tissues and cause inflammatory changes. Taken together, PGRN might be involved in lipid metabolism and adipose tissue inflammation, leading to the pathogenesis of metabolic syndrome.

Poster Session

THE EFFECTIVENES OF EZETIMIBE PLUS PRAVASTATIN FOR HYPERCHOLESTEROMIC PATIENTS WITH POSTPRANDIAL HYPERLIPIDEMIA

Yasunori Sawayama, H. Ohnishi, T. Koga, M. Kainuma, N. Furusyo, J. Hayashi

Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan

Background and objectives: Postprandial hyperlipidemia is known to be a high-risk factor for atherosclerotic disease. It is unknown whether the addition of ezetimibe to statin therapy affects postprandial hyperlipidemia. To compare the effects of postprandial hyperlipidemia treated by esetimibe 10mg alone or esetimibe 10mg plus pravastatin 10mg.

Methods: This study was an open-label, blinded-to-endpoint study. Of the 110 hypercholesterolemic patients (LDL-C≥ 120 mg/dl) screended by meal test, 50 postprandial hyperlipidemic patients were randomized (n=25 assigned to ezetimebe alone and n=25 assigned to ezetimebe plus paravastatin). The 2-h meal test, which consisted of 115 g of cookies (energy 592 kcal; glucose 75 g; protein 8 g; fat 28.5 g), was conducted before and after the six months of study. A diagnosis of postprandial hyperlipidemia was made when ⊿TG(0-2h) was over 66 mg/dl.

Results: After six months of treatment , mean ⊿TG(0-2h) was significantly reduced by 29mg/dl and 36mg/dl , respectively in the group receiving ezetimibe alone and ezetimibe plus pravastatin (p=0.0025, p=0.0006). Mean LDL-C was reduced by 15mg/dl and 46mg/dl, respectively in the groups receiving ezetimibe alone and ezetimibe plus pravastatin (p=0.0039, p=0.0006).

Conclusions: This study demonstrated that ezetimibe confers a beneficialeffect on lipid metabolism, especially in combination with pravastatin in the postprandial state. These data confirm that a combination therapy that simultaneously inhibits both cholesterol biosynthesis and intestinal cholesterol absorption represents the most effective cholesterol-lowering intervention.

Poster Session

CHOLESTEROL LOADING INDUCES SMOOTH MUSCLE CELLS TRANSDIFFERENTIATION TO MACROPHAGE-LIKE CELLS

A. Bonomo, M. Canavesi, I. Giunzioni, C. Anelli, A. Corsini, **Stefano Bellosta**

Department of Pharmacological Sciences, University of Milan, Milan, Italy

Macrophages accumulate cholesterol and cholesteryl esters (CE) becoming foam cells (FC). However, some FC may originate from smooth muscle cells (SMCs). Rong (PNAS 2003) showed that cholesterol-loaded SMCs rapidly assume a FC-like appearance and lose the expression of SMC markers. To further characterize the transdifferentiation of SMCs to macrophages, aortic mouse SMCs were isolated from C57/BL6 mice and loaded with free cholesterol complexed to methyl-beta-cyclodextrin. After cholesterol loading, SMCs lost the expression of SMC markers, such as myosin heavy chain and alpha-actin (- 33% and - 65%, respectively), and rapidly assumed a FC appearance with Oil Red O-stainable lipid droplets. Cellular total cholesterol content increased 2-3 fold, due to a 2.5 fold increase in the activity of the esterifying enzyme ACAT, which could be inhibited by an ACAT inhibitor or by HDL. Cholesterol loading of SMC enhanced the expression of the macrophage marker Mac-2 (11-fold), of ABCA1 (3-4 fold, both at the protein and mRNA levels) and the production of nitric oxide, while the expression of ICAM-1 and VCAM-1 was not affected. Also the expression of several matrix metalloproteinases (MMP) mRNA was affected: MMP-1 (+30% vs control), MMP-2 (+98%), MMP-3 (+95%), MMP-9 (+ 283%), and of the tissue inhibitor of MMP (TIMP-1, +185%). Interestingly, the addition of HDL reduced both the stimulation of ABCA1 and MMP-9, and the inhibition of alpha-actin expression. These results indicate that cholesterol loaded SMCs may transform into macrophage marker-positive FC with some pro-atherogenic features. HDL seems to play a protective role against this process.

Poster Session

LOW LEVELS OF CIRCULATING SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS IN PATIENTS WITH DEGENERATIVE AORTIC VALVE STENOSIS AND CARDIOVASCULAR CALCIFICATIONS

Annamaria Mazzone[1], A.I. Corciu[2], A. Vianello[1], S. Del Turco[2], I. Foffa[1], T. Navarra[2], D. Chiappino[1], S. Berti[1], G. Basta[2]

[1]Heart Hospital , G.Monasterio Foundation /CNR, Massa, [2]IFC/CNR, Pisa, Italy

Background and aim: It has been suggested that atherosclerotic mechanisms are involved in the pathogenesis of aortic valve stenosis (AVS). We hypothesised that low levels of the soluble receptor for advanced glycation and products (sRAGE) might be associated with AVS due to its clinical and pathological associations with atherosclerosis.

Methods: We enrolled 75 consecutive patients with severe AVS scheduled for surgical aortic valve replacement and 39 controls without AVS matched for age and gender. Besides the traditional risk factors, we evaluated plasma levels of the soluble Receptor for Advanced Glycation End products (sRAGE), and C-reactive protein (CRP). All underwent transthoracic echocardiography, carotid arteries ultrasound scan and coronary angiography. The aortic and coronary calcium by Multislice Computed Tomography was assessed.

Results: The values of sRAGE were significantly lower ($p < 0.01$) in AVS (124 [37-497] pg/mL, median [interquartile range]) than in controls (306 [155-637] pg/mL), while the CRP was significantly higher ($p < 0.05$) in AVS (0.3 [0.1-1.07] mg/dL) than in controls (0.1 [0.1-0.37] mg/dL). In AVS the sRAGE correlated inversely with age ($r = -0.355$, $p = 0.0002$) cholesterol ($r = -0.228$, $p < 0.05$) and coronary calcification ($r = -0.570$, $p = 0.0087$). In all subjects we found an inverse correlation between circulating sRAGE and the number of echographically assessed sites of calcification (ANOVA, $p < 0.0001$).

Conclusion: Since sRAGE could exert antiatherogenic effects by preventing inflammatory responses mediated by cell surface RAGE activation, low levels in AVS patients indicate that ligand-RAGE axis may contribute to AVS and cardiovascular calcifications pathogenesis.

Poster Session

CHANGES IN PROTEASOMAL ACTIVITY AT MODELING OF CHOLESTEROL-INDUCED ATHEROSCLEROSIS: QUERCETIN INFLUENCE

Dennis Pashevin[1], L. Tumanovskaya[2], V. Dosenko[1], A. Moibenko[1]

[1]General and Molecular Pathophysiology, Institute of Physiology, [2]General and Molecular Patophysiology, Bogomoletz Institute of Physiology, Kiev, Ukraine

We studied changes of proteasomal activity in cholesterol-induced atherosclerotic lesions. Also we provide evidence that quercetin inhibits proteasomal activity in circulating leucocytes after intravenous application to control animals and significantly prevents increase of proteasomal activities in aorta and decrease the intensity of atherosclerotic lesions at application on rabbits with high-cholesterol diet. Moreover, mentioned chemicals did not influence on level of cholesterol, triglycerides and lipoproteins. Experiments were carried out on 30 rabbits divided into three groups: control animals, rabbits that every day received forage enriched by cholesterol (1 % cholesterol diet) for 4 weeks and animals that received the same cholesterol-rich diet with application of quercetin. Changes in proteasomal activity at modeling of cholesterol-induced atherosclerosis in rabbits are presented: trypsin-like activity (TL) of proteasome is increased in aortic tissues in 2,4 times (P0.05), and only PGPH is significantly increased in isolated lymphocytes (4-folds compared with control, P

Poster Session

LENTIVIRAL OVEREXPRESSION OF HYALURONIC ACID SYNTHASES 1 AND 2 RESTORES ESTRADIOL-MEDIATED INHIBITION OF PDGF-INDUCED PROLIFERATION IN VASCULAR SMOOTH MUSCLE CELLS

Till Freudenberger[1], G. Dai[1], P. Mayer[2], H.-K. Heim[2], J.W. Fischer[1]

[1]Institut für Pharmakologie, Uniklinikum Essen, Universität Duisburg-Essen, Essen, [2]Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn, Germany

Backround: Hyaluronic acid (HA), a constituent of the extracellular matrix, is produced by the three HA-synthase isoforms HAS1, HAS2 and HAS3. Platelet-derived growth factor-BB (PDGF-BB) induces HA synthesis in vascular smooth muscle cells (SMC) and thereby increases their proliferative capacity while estradiol inhibits the PDGF-BB-induced proliferation of SMCs. However, nothing is known about the effects of estradiol on HAS-expression and HA-secretion.

Methods: Human SMCs were serum-deprived and treated with PDGF-BB (10 ng/ml) alone or in combination with estradiol ($1x10^{-9}$ M and $0.1x10^{-6}$ M). HAS-mRNA-expression, HA-secretion and proliferation were investigated. Furthermore, human SMCs were infected with lentiviral vectors to overexpress HAS1- and HAS2-isoenzymes, serum-deprived and stimulated as described above. Proliferation and ERK-kinase phosphorylation were determined.

Results: Estradiol significantly reduced the PDGF-induced expression of HAS1-mRNA. PDGF-induced HAS2-mRNA expression was reduced to 79 % ± 6% with $0.1x10^{-6}$ M estradiol ($n = 7$, $p < 0.05$). HA-secretion decreased to less than 38% in the presence of estradiol. Furthermore, proliferation of SMCs treated with PDGF and estradiol decreased concentration-dependently. Of note, lentivirally mediated overexpression of either HAS1- or HAS2-isoenzyme abolished the estradiol-mediated decrease of the PDGF-BB-induced proliferation of SMCs. Along with this the pERK/tERK ratio that was decreased by estradiol in SMCs treated with PDGF-BB and increased to control levels after overexpression of either HAS1 or HAS2.

Conclusion: For the first time we provide strong evidence that the estradiol-mediated decrease in the PDGF-BB induced proliferation in SMCs is at least in part due to reduced HAS1- and HAS2- mRNA expression levels.

Poster Session

THE EFFECTS OF MYOCYTE ENHANCER FACTOR 2A GENE ON PROLIFERATION, MIGRATION AND PHENOTYPE OF VASCULAR SMOOTH MUSCLE CELLS

Wang Zhao, S.-P. Zhao, P.-C. Wu, D.-Q. Peng

Cardiology Department, Second Xiangya Hospital of Central South University, Changsha, China

Aims: Synthetic vascular smooth muscle cells (VSMCs) tend to proliferate and migrate more facilely than contractile ones in atherosclerosis. But the genetic basis for VSMCs phenotypic switching is unclear. Recent studies showed that myocyte enhancer factor 2A(MEF2A) 21-base pair deletion mutation (Δ21, dominant negative mutation) could be an inherited marker for CHD. We hypothesis that this mutation affect the VSMCs phenotypic switching.

Methods: Human aortic VSMCs were used. Three groups VSMCs transfected MEF2A wild-type(WT) plasmid, MEF2A Δ21 plasmid or MEF2A siRNA were studied. The changes of VSMCs proliferation was determined by MTT and VSMCs migration was measured by Millicell chamber. The expression of MEF2A protein, α-SM-actin, SM22α, osteopontin and MAPK signaling pathway were detected by western blotting.

Results: MEF2A siRNA successfully knockdown MEF2A protein. MEF2A protein was overexpressed in VSMCs transfected either with MEF2A WT or Δ21 plasmid. Compared to MEF2A WT group, MEF2A Δ21 and siRNA groups showed higher VSMCs proliferation (MTT 0.66 vs 0.32, $P< 0.01$) and more migration (58 vs 21, $P< 0.01$). In addition, western blotting confirmed that MEF2A Δ21 and siRNA could induce downregulation of α-SM-actin and SM22α ($P< 0.01$) and upregulation of osteopontin ($P< 0.01$). The phosphorylated p38 signaling pathway expression was significantly enhanced in the MEF2A Δ21 and siRNA groups as compared to MEF2A WT transfection ($P< 0.01$).

Conclusions: These results suggest that MEF2A dominant negative mutation and RNA silence could induce phenotype switching of VSMCs, leading to its increased proliferation and migration. p38 MAPK signaling pathway may participate in it.

Poster Session

THE CELLULAR THIOL REDOX STATE DETERMINES MONOCYTE RESPONSIVENESS TO CHEMOATTRACTANTS AND REGULATES MACROPHAGE RECRUITMENT INTO ATHEROSCLEROTIC LESIONS

Q. Zhao[1], S. Ullevig[2], **Reto Asmis**[1,2]

[1]Department of Clinical Laboratory Sciences, [2]Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Monocyte diapedesis and macrophage recruitment are critical processes in the development and progression of atherosclerotic lesions. Previously, we showed that the thiol redox state of macrophages is a strong predictor of both macrophage recruitment and atherosclerotic lesion formation in metabolically-challenged mice. We now provide evidence that increased macrophage recruitment is mediated by protein-*S*-glutathionylation and the result of increased monocyte responsiveness to chemoattractants. Prolonged exposure of THP-1 monocytes to human LDL, high glucose concentrations (HG) or LDL+HG increased intracellular H_2O_2 formation, protein *S*-glutathionylation, and increased chemotaxis in response to MCP-1 and PDGF-BB. Exogenously added H_2O_2 at levels that mimicked intracellular H_2O_2 generation induced by LDL or HG, also promoted protein-*S*-glutathionylation and increased monocyte chemotaxis in response to MCP-1 and PDGF-BB. Overexpression of glutaredoxin 1 did not affect monocyte chemotaxis induced by either MCP-1 or PDGF-BB, but prevented H_2O_2-induced protein-*S*-glutathionylation and completely protected against the sensitizing effects of H_2O_2 on monocyte chemotaxis. Exposure of THP-1 monocytes to LDL+HG resulted in the upregulation of Nox4, a novel Nox family member we recently identified in human blood monocytes and macrophages, suggesting that Nox4 may be the source of intracellular H_2O_2 generated in metabolically-stressed monocytes. Induction of hypercholesterolemia and hyperglycemia in C57BL/6 mice promoted protein-*S*-glutathionylation in macrophages and increased macrophage chemotaxis into MCP-1-loaded Matrigel plugs implanted subcutaneous in these mice. Macrophage chemotaxis strongly correlation with levels of protein-*S*-glutathionylation (r^2=0.789, P< 0.001). Our data support a novel mechanism linking metabolic disorders to altered thiol-redox signaling in monocytes and increased macrophage recruitment to sites of tissue injury.

Poster Session

HYPOXIA UP-REGULATES FIBULIN-5 IN ENDOTHELIAL CELLS THROUGH A HIF-1 DEPENDENT MECHANISM

Anna Guadall, R. Rodríguez-Calvo, R. Aledo, O. Calvayrac, J. Martínez-González, C. Rodríguez

Centro de Investigación Cardiovascular.CSIC-ICCC. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Introduction: Fibulin-5 (FBLN5) is an extracellular matrix protein involved in vascular remodelling that controls endothelial cell (EC) adhesion, motility and proliferation.

Objective: To analyze FBLN5 regulation by hypoxia in EC.

Methods: Studies were conducted in human umbilical venous EC (HUVEC) and in bovine aortic EC (BAEC). FBLN5 and HIF-1α levels (mRNA and protein) were analyzed by real time-PCR and Western blot, respectively, and transcriptional activity by transient transfection analysis. EMSA and chromatin immunoprecipitation (ChIP) assays were also performed.

Results: Hypoxia (1% O_2) increased FBLN5 mRNA levels in EC in a time-dependent manner. Maximal induction (about 2.5 fold) was achieved after 24-48 h under hypoxic conditions. The increase in FBLN5 mRNA levels observed in hypoxic cells was blocked by pretreatment with inhibitors of the PI3K/Akt/mTOR axis (LY294002 and rapamycin) and mimicked by dimethyl oxal glycine, that prevents proline hydroxylase-mediated degradation of HIF1α. Interestingly, silencing of HIF1α completely prevented the increase of FBLN5 expression observed under hypoxic conditions. Accordingly, both hypoxia and HIF-1α over-expression increased FBLN5 transcriptional activity. Serial deletion studies revealed the involvement of element/s located in the proximal promoter region and in silico studies identified a putative hypoxia response element (HRE). Mutation of this sequence completely abrogated the hypoxia-mediated induction of FBLN5 promoter activity. Furthermore, EMSA and ChIP assays demonstrated increased HIF-1 binding to the FBLN5 HRE site under hypoxic conditions.

Conclusions: HIF-1 signalling underlies the increase of FBLN5 expression elicited by hypoxia in EC. FBLN5 induction by hypoxia could contribute to the adaptive response of EC to ischemia.

Poster Session

LDL UP-REGULATES CCL20 IN HUMAN VASCULAR SMOOTH MUSCLE CELLS THROUGH A NF-KB DEPENDENT MECHANISM

Olivier Calvayrac, R. Rodríguez-Calvo, M. Gentile, A. Guadall, C. Rodríguez, J. Martínez-González

Centro de Investigación Cardiovascular. CSIC-ICCC. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Introduction: CCL20 (MIP-3α, *Macrophage Inflammatory Protein-3α*) is a chemokine involved in immune response, integrin activation, and cytoskelenton reorganization, among other processes.

Objective: To analyze CCL20 regulation by low-density lipoproteins (LDL) in human vascular smooth muscle cells (VSMC).

Methods: VSMC were isolated from human coronary arteries and LDL from plasma of healthy volunteers. CCL20 mRNA levels were analyzed by real time-PCR. Signalling pathways were analyzed using specific inhibitors. Transcriptional activity of CCL20 promoter was analyzed by transient transfection assays using luciferase reporter plasmid, and binding of NF-κB by electrophoretic mobility shift assay (EMSA).

Results: LDL increased CCL20 mRNA levels in VSMC in a dose and time-dependent manner. Maximal induction (about 4-fold) was achieved when VSMC were exposed to LDL (300 µg cholesterol/ml) for 4 h. The increase in CCL20 mRNA levels observed was blocked by DRB, suggesting a transcriptional regulation. Pretreatment with BAPTA-AM (a calcium chelator), GF109203X (a PKC inhibitor), pertusis toxin (a GPCR inhibitor) or inhibitors of MAPK pathways (SB203580 and U0126) also prevent CCL20 induction by LDL. Interestingly, pretreatment with parthenolide (a NF-kB inhibitor) completely prevented LDL-induced CCL20 up-regulation. LDL increased CCL20 promoter activity. *In silico* studies identified a putative NF-κB response element in CCL20 promoter. Mutation of this response element completely abrogated the LDL-mediated induction of CCL20 promoter activity. Finally, the increased binding of NF-κB to this response element in response to LDL, observed by EMSA, was prevented by parthenolide.

Conclusions: NF-κB signalling underlies the increase of CCL20 expression elicited by LDL in human VSMC.

Poster Session

OXIDIZED LDL EXPOSURE DECREASES KRÜPPEL-LIKE FACTOR 2 EXPRESSION AND INCREASES PRO-INFLAMMATORY CAPACITY OF M2 MACROPHAGES

Berry van Tits, R. Stienstra, M. Netea, L. Joosten, A. Stalenhoef

Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

We recently reported that exposure to oxidized (ox) LDL increases expression of pro-inflammatory cytokines IL-6 and IL-8 and decreases expression of anti-inflammatory IL-10 of M2 macrophages stimulated with LPS. To address involvement of the transcription factors PPARγ, LXRα and KLF2 in the altered inflammatory responses, we measured relative mRNA expression of these factors in M1 and M2 macrophages pre and post oxLDL exposure (50 µg/ml, 24 hours). Pre oxLDL exposure, expression of KLF2 was higher (p=0.03) and PPARγ lower (p=0.006) in M2 macrophages versus M1 macrophages. Expression of LXRα did not differ between both phenotypes. Upon oxLDL exposure, in M1 macrophages expression of PPARγ decreased, no changes were found for LXRα and KLF2. In M2 macrophages oxLDL-loading did not change PPARγ, but LXRα increased and KLF2 decreased. Spearman's correlation revealed no correlations of KLF2, PPARγ and LXRα mRNA with LPS-induced expression of IL6, IL8, or IL-10 (mRNA or protein) in M1 macrophages. In M2 macrophages, we also found no correlations for PPARγ and LXRα. However, KLF2 correlated with LPS-induced production of IL-6 (r=-0.80, p=0.002), IL-8 (r=-0.73, p=0.007) and IL-10 (r=0.60, p=0.04). Moreover, KLF2 also correlated with LPS-induced mRNA expression of IL-10 (r=0.66, p=0.02) and borderline significancy was obtained for correlation with LPS-induced mRNA expression of IL-6 (r=-0.53, p=0.07). These correlations indicate that KLF2 may downregulate inflammatory signaling in M2 macrophages. To specifically address a causal role of KLF2 in regulation of inflammatory capacity of macrophages, siRNA experiments to knock down KLF2 will be performed.

Poster Session

NPY RECEPTORS ON T-REGULATORY CELLS DERIVED FROM CAROTID PLAQUES OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS WITH CAROTID STENOSIS

Devendra Agrawal[1], D. Pankajakshan[1], G. Jia[1], Z. Shao[1], I. Pipinos[2]

[1]Clinical & Translational Science, Creighton University School of Medicine, [2]Vascular Surgery, University of Nebraska Medical Center, Omaha, NE, USA

Naturally occurring $CD4^+CD25^+$ T-Regulatory cells (T-Regs), which actively maintain immunological tolerance to self and non-self antigens, are powerful inhibitors of atherosclerosis. Here, we examined the density of natural ($CD4^+CD25^+Foxp3^+$) and inducible ($CD4^+CD25^-Foxp3^-$) T-Regs in plaques of symptomatic(S) and asymptomatic (AS) patients with carotid stenosis. The two subsets of T-Regs were isolated from S- and AS-plaques by enzyme digestion followed by FACS. The $CD4^+CD25^+$ cells were positively selected (purity>97%) from enriched $CD4^+$ T cell fraction using $CD4^+CD25^+$ Regulatory T-cell isolation kit and autoMACS (MiltenyiBiotech, Germany). The remaining $CD4^+CD25^-$ cells (purity>98%) were collected. The percentage of $CD4^+CD25^+Foxp3^+$ cells in AS and S plaques were quantified by flow cytometry. The mRNA expression of NPY-Y2 and NPY-Y5 receptors was examined by qPCR. Six-to-8-fold higher density of $CD4^+CD25^+$ T-Regs was observed in carotid plaques of AS than S patients, whereas $CD4^+CD25^-$ cells were 3-4 times more in the plaques of S than AS patients. The $Foxp3^+$ T-Regs in total $CD4^+CD25^+$ cells were significantly higher (75.8%) in AS-plaques than S-plaques (51.4%). There was no expression of NPY-Y1 in either T-Regs population. The $CD4^+CD25^+$ T-Regs from S-plaques showed higher NPY-Y2 expression levels than AS. The NPY-Y5 expression levels were similar in S and AS $CD4^+CD25^+$ T-Regs. The $CD4^+CD25^-$ cells derived from S plaques showed approximately 10-fold and 5-fold increase in NPY-Y2 and NPY-Y5 receptors, respectively, when compared to AS-plaques. These results suggest the potential regulatory role of NPY-Rs in natural and inducible T-Regs in regulating the stability of plaques in atherosclerosis. (Supported by NIH grant R01-073349).

Poster Session

RHOA AND TCTA - A "BACK-TO-BACK" PROMOTER SYSTEM

Bianca Schröer[1], R.-G. Telgmann[1], F. Bruns[1], E. Brand[2], S.-M. Brand-Herrmann[1]

[1]Department of Molecular Genetics of Cardiovascular Disease, Leibniz-Institute for Arteriosclerosis Research, [2]Department of Internal Medicine D, Nephrology and Hypertension, University Hospital Münster, Münster, Germany

Background and aims: The small GTPase RhoA is pathophysiologically involved in inflammatory disease and according to recent GWA studies it plays a prominent role in cardiovascular disease (CVD). *RhoA* and *TCTA* are located on chromosome 3p21.3 in a "back-to-back" orientation and separated by a small 112 bp promoter region, containing two TATA boxes. We herein aimed at molecular genetically characterize the *RhoA*/*TCTA* promoter regions.

Material and methods: We directly sequenced the 112 bp *RhoA/TCTA* promoter region in 120 chromosomes of high-risk CVD patients (MolProMD Study). A series of promoter deletion constructs (n=4 [-1372/-1; -948/-1; -490/-1; -100/-1]) were generated using the pGL3-reporter gene system followed by transfection into EA.hy926 cells. Genetic variants and TATA box deletions were introduced by site-directed mutagenesis.

Results: We identified a common nucleotide substitution in the *RhoA*/*TCTA* promoter region, which alters one of the TATA boxes. All *RhoA* and *TCTA* promoter deletion constructs exerted sufficient transcriptional activity. However, the promoter constructs containing the wildtype TATA box were significantly more active in EA.hy926 than the promoter constructs containing the altered TATA box ($P < 0.0001$). Serially introduced mutations in the promoter portion showed that for sufficient transcriptional activity of RhoA, both TATA boxes are needed in their non-mutated constellations and with unchanged nucleotide spacing between them.

Conclusion: We were able to show that RhoA and TCTA both utilize the shared 112 bp promoter region in EA.hy926 cells and that meaningful transcriptional activity clearly depends on the presence of non-variant nucleotide composition in that particular promoter portion.

Poster Session

LDL INCREASES VASCULAR ET_{B2} RECEPTOR EXPRESSION VIA ACTIVATION OF ERK1/2 AND P38 MAPK PATHWAYS

Yaping Zhang, J.-P. Zheng, W. Zhang, L. Edvinsson, C.-B. Xu

Division of Experimental Vascular Research, Institute of Clinical Science in Lund, Lund University, Lund, Sweden

In cardiovascular disease, there are increased expression of endothelin type B_2 (ET_{B2}) receptors on the vascular smooth muscle cells (VSMC) and enhanced ET_{B2} receptor-mediated VSMC contraction, which contribute to the pathogenesis of cardiovascular disease. The present study was designed to investigate if cardiovascular risk factor low density lipoprotein (LDL) could cause the increased ET_{B2} receptor expression and if intracellular mitogen-activated protein kinases (MAPK) were involved in this process. Rat mesenteric arteries were isolated and cut into ring segments. The segments were then incubated at 37°C, 5% CO_2 with different concentrations (50, 100 and 200mg/L) of LDL for up to 48 hrs. Effect of LDL on the ET_{B2} receptor expression was examined at functional contraction (in vitro myograph), mRNA (real-time PCR), and protein (Western blot) levels. The involvement of intracellular MAPK pathways was studied by using Western blot and specific inhibitors for extracellular signal-regulated kinases (ERK1/2) and p38 MAPK. Our results show that LDL increased the ET_{B2} receptor-mediated vasoconstriction and mRNA expression for VSMC ET_{B2} receptors in concentration (50, 100 and 200 mg/L) and time (6, 12, 24 and 48 hrs) dependent manners. LDL activated ERK1/2 and p38 MAPK on the VSMC. The special inhibitors for ERK1/2 (PD98059 and U0126) and p38 (SB203580 and SB239063) MAPK abolished the LDL effects. In conclusion, we have demonstrated for the first time that LDL increases VSMC ET_{B2} receptor expression through activation of ERK1/2 and p38 MAPK pathways. Understanding the intracellular MAPK signal mechanisms may provide new strategies for treatment of cardiovascular disease.

Poster Session

PROFILING OF MICRORNAS FOLLOWING VASCULAR INJURY IN APOE$^{-/-}$ MICE

Maliheh Nazari Jahantigh, C. Weber, A. Schober

Institute for Molecular Cardiovascular Research/RWTH Aachen University, Aachen, Germany

Objective: We aimed to identify differentially regulated microRNAs after vascular injury in hyperlipidemic mice.

Methods: Wire-induced carotid injury was performed in ApoE$^{-/-}$ mice on western-type diet. Uninjured arteries served as control. RNA was isolated after 1, 7, 14, and 28 days (n=3-4/group) and hybridized to an Agilent microRNA microarray (Sanger v12). Significantly regulated microRNAs (>2-fold) over time ($P< 0.05$; ANOVA and Benjamini-Hochberg correction) were clustered by K-means algorithm. QRT-PCR was performed for validation.

Results: 95 out of 611 microRNAs were differentially expressed and seven groups were separated by cluster analysis. Although all microRNAs in group 1-3 were increased from day 7 on, expression at day 1 was unchanged or decreased in group 1 (e.g. miR-155) and 2 (e.g. miR-199a-3p), respectively, or slightly up-regulated in group 3 (e.g. miR-21). Group 4 (e.g. miR-188-5p) was characterized by highest expression from day 1 on. Following down-regulation at 1 day, microRNAs in group 5, such as let-7i, were elevated again to control levels thereafter. In contrast, persistent down-regulation as early as day 1 was specific for group 6 (e.g. miR-378). The expression of microRNAs in group 7 (e.g. miR-129-3p), however, was not significantly different from control at day 1, but declined predominantly after 7 and 14 days. The expression profile of 6 miRs from different groups was verified by qRT-PCR.

Conclusion: Distinct groups of similarly regulated microRNAs were detected in the course of neointima formation in hyperlipidemic mice. These groups might be functionally related to specific cellular and molecular processes of vascular wound healing.

Poster Session

HYPERCHOLESTEROLEMIA (HC) ABROGATES THE CONCENTRATION-DEPENDENT BIPHASIC EFFECT OF ANGIOTENSIN (ANG II) ON ENDOGENOUS CHOLESTEROL SYNTHESIS IN HUMAN MONOCYTES

Gabriella Fóris[1], Z. Sarang[2], I. Seres[1], G. Kónya[1], G. Paragh[1]

[1]First Department of Medicine, [2]Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

Objective: Ang II the multifunctional hormone regulates cholesterol biosynthesis in human monocytes. Our aim was to elucidate the concentration dependence of the Ang II effect in monocytes derived from control and HC patients, and their downstream signalling.

Methods: Control and HC monocytes stimulated with Ang II in the presence or absence of different inhibitors.

Results: Our results are as follows: A concentration dependent biphasic effect could only be detected in control monocytes whereas in cells from HC patients only elevated cholesterol synthesis was found. The signal pathway of 10 nM Ang II stimulation involves Ca^{2+} signal, activation of PI3K, MAPK and HMG CoA reductase. In the 500 nM Ang II stimulated control monocytes the suppression of cholesterol synthesis was dependent on the Ca^{2+} signal, the H-7 sensitive cPKC and PI3K, whereas in monocytes from HC patients only PI3K was involved in increased cholesterol synthesis.

Conclusion: We conclude that Ang II signalling in control monocytes leads through PLC - Ca^{2+} translocation - conventional PKC- SOCS3 pathway. In contrast, in monocyes of HC patients is characterized by Ca^{2+} influx, abrogation of H-7 sensitive cPKC, and by PI3K- Ins(345)P3 dependent ξPKC isoform - NFκB - HMG CoA reductase activation.

Poster Session

EVIDENCE FOR INFILTRATION OF NEUTROPHIL GRANULOCYTES IN PLAQUES OF UNSTABLE CAROTID ARTERY DISEASE

Bernhard Dorweiler[1], M. Torzewski[2], M. Dahm[1], C. Kirkpatrick[3], K. Lackner[2], C. Vahl[1]

[1]Cardiothoracic & Vascular Surgery, [2]Laboratory Medicine, [3]Pathology, University Medical Center, Mainz, Germany

Objective: To assess the role of neutrophil infiltration in plaque specimen of stable and unstable carotid artery disease.

Methods: Plaque specimen from patients with stable and unstable carotid artery disease were obtained during surgical carotid artery desobliteration. Samples were processed for immunohistochemistry and analyzed for markers of apoptosis, macrophages, granulocytes and elastase.

Results: 38 specimen from patients with stable (19) and unstable (19) carotid artery disease were formalin-fixed, paraffin embedded and analyzed by histology and immunohistochemistry. Patient groups were not significantly different for cardiovascular risk factors, degree of stenosis (mean 87% for stable and 85% for unstable disease) and statin/antiplatelet medication. Interestingly, neutrophil infiltration was observed in 5 cases ($p < 0.05$) of the group with unstable disease, whereas none could be detected in stable disease. Infiltration of neutrophil granulocytes colocalized with markers of plaque vulnerability, i.e. macrophage infiltration (CD68+), apoptosis (TUNEL+) and elastase.

Conclusion: Using a 3D in vitro model of human neo-intima, we previously identified a possible recruiting mechanism for granulocytes in atherosclerotic plaques that was paralleled by secretion of matrixdegrading enzymes (elastase). In this study, we were able to present a novel finding of neutrophil infiltration in plaques of unstable carotid artery disease. Taken together, those results corroborate the currently discussed active role of neutrophil granulocytes in plaque destabilization.

Poster Session

RELATIONSHIP BETWEEN CD59 POSITIVE PERIPHERAL MONONUCLEAR CELLS AND HDL-CHOLESTEROL LEVELS IN PATIENTS WITH PROGRESSING ATHEROSCLEROSIS

Victor Gurevich[1], L. Vasina[2], A. Lugovaya[2], N. Petrischev[3]

[1]Center of Atherosclerosis and Lipid Disorders, Mechnikov's State Medical Academy, [2]Clinical Chemistry, Sokolov's Hospital, [3]Chair of Pathology, Pavlov's State Medical University, Saint Petersburg, Russia

Aim: It has been recently experimentally shown that one of the anti-atherogenic mechanisms of high-density lipoproteins (HDL) is their complement inhibiting activities. The aim of this investigation was to examine the relationships between the expression of protectin CD59 - lipid-anchored inhibitor of complement lysis that is expressed on the membranes of blood cells and HDL-cholesterol levels in patients with progressing atherosclerosis.

Methods: Blood samples of 87 (53 male and 34 female) patients with clinical and instrumental signs of acute coronary syndrome and blood samples of 43 volunteers as controls were examined in this study**.** The control group was corresponding on average age, sex and lipid profile however with no or minimal signs of atherosclerosis progression. **Flow cytometry** with fluorescently labeled monoclonal antibodies was used to determine CD59 (+) peripheral mononuclears. HDL-cholesterol levels were measured enzymatically.

Results: It has been shown that mean quantity of CD59(+) cells was significantly higher in group with documented progression of atherosclerosis in comparison with control group. In patients with transmural myocardium infarction the expression of CD59 (+) was more intensive then in those with unstable angina (60,6±8,4% и 41,8±9,4% respectively). The positive correlation (r = 0.610) between HDL-cholesterol levels and quantity of cells expressed CD59 antigen has been demonstrated.

Conclusion: Our findings specify that association of HDL-cholesterol levels with CD59 expression is the first evidence for protective role of HDL in human complement-mediated atherogenic injury.

Poster Session

PROTEOMIC ANALYSIS OF HUMAN ATHEROSCLEROTIC CORONARY INTIMA REVEALS NOVEL POTENTIAL BIOMARKERS IN HUMAN ATHEROSCLEROSIS

F. de la Cuesta[1], G. Alvarez-Llamas[1], I. Zubiri[1], A.S. Maroto[1], A. Donado[2], L.R. Padial[3], A. G. Pinto[2], M.G. Barderas[4], **Fernando Vivanco**[1,5]

[1]Immunology, Fundacion Jimenez Diaz, [2]Cardiac Surgery, Hospital Gregorio Marañon, Madrid, [3]Cardiology, Hospital Virgen de la Salud, [4]Vascular Physiopatology Laboratory, Hospital Nacional de Paraplejicos, Toledo, [5]Biochemistry and Molecular Biology, Universidad Complutense, Madrid, Spain

Introduction: Tissue proteomic studies on atherosclerosis have been traditionally focused on whole artery extracts. Atherogenesis initiates within the intima and implies circulating inflammatory cells recruitment together with vascular smooth cells coming from the media. These events may affect the proteomic profile of the layer during atherosclerosis development.

Methods: Coronary and radial (control) samples from bypass surgery and atherosclerotic and preatherosclerotic (control) coronaries from necropsy origin were immediately washed in saline and frozen embedded with OCT. Intima was isolated by Laser Microdissection and Pressure Catapulting (LMPC) with a Microbeam System (PALM Microlaser). After an optimized protocol of protein extraction [1] samples were analyzed by saturation labeling DIGE (GE Healthcare). A subset of altered proteins was validated by immunohistochemistry (IHC) using additional coronary and radial specimens.

Results: Seven proteins were found overexpressed by atherosclerotic coronary intimas, while 5 were down-regulated. Two of these proteins were validated by IHC on an independent group of arteries.

Conclusions: Coronary intima protein profile varies with atherosclerosis progression.

Here we describe 12 proteins altered in atherosclerotic coronary intima that may have a potential utility as biomarkers of coronary atherosclerosis.

[1] F. de la Cuesta, G. Alvarez-Llamas, AS. Maroto, A. Donado, R. Juarez-Tosina, L. Rodriguez-Padial, AG. Pinto, MG. Barderas and F. Vivanco. An optimum method designed for 2-D DIGE analysis of human arterial intima and media layers isolated by laser microdissection *Proteomics Clin. Appl*. 2009, 3, 1174-1184

Poster Session

PROTEOMIC CHARACTERIZATION OF EXTRACELLULAR ENVIRONMENT COMPONENTS REVEALS NOVEL PROTEINS IN THE HUMAN AORTA

Athanasios Didangelos[1], K. Mandall[2], M. Jahangiri[3], M. Mayr[1]

[1]British Heart Foundation, James Black Center, King's College London, London, UK, [2]John Hopkins University, Baltimore, MD, USA, [3]St. George's Healthcare NHS Trust, London, UK

Although proteomics has been previously applied to vascular tissues, few studies have specifically targeted the proteins of the extracellular environment. Thus, its detailed composition remains to be characterized. In this study, our objective was to develop a methodology for the extraction and enrichment of extracellular proteins from human aortas. We designed a biochemical subfractionation which achieved minimum contamination with cellular proteins and very effective solubilisation of the proteins of the extracellular environment.

Our methodology resulted in the identification by state-of-the-art mass spectrometry of 110 extracellular proteins, of which one third have never been reported in the proteomic literature of vascular tissues so far. In particular, our study revealed the presence of 4 novel glycoproteins in human aortas (podocan, sclerostin, agrin and asporin) and we were able to localize them on the aortic ECM. Their function in the vasculature is currently unknown. Interestingly, we found that cholesterol loading regulates podocan and agrin expression in human aortic smooth muscle cells. More detailed studies are currently in progress.

Finally, our methodology allowed us to screen for pathological proteolysis in the aortic extracts. We were able to accurately detect and confirm extensive degradation of fibronectin and relate it to the presence of MMP-9. In conclusion, we expect this proteomic methodology to further our understanding in the composition of the extracellular environment, shed light on ECM remodelling and degradation and provide insights into important pathological processes, such as plaque rupture, aneurysm formation and restenosis.

Poster Session

EPIGENETIC MODIFICATIONS IN HUMAN CAROTID ATHEROMATOUS PLAQUES

C. Kroupis[1], S. Georgopoulos[2], N. Poumpouridou[1], S.C. Oliveira[1], I. Chatziioannidou[1], **Amalia Dionyssiou-Asteriou**[1]

[1]Attikon University Hospital, University of Athens Medical School, Haidari, [2]Vascular Unit, First Surgical Dept, University of Athens Medical School, Athens, Greece

Objective: DNA promoter hypermethylation is the most common epigenetic mechanism and responsible for loss of critical gene expression. There is increasing evidence that DNA methylation plays a significant role in many pathways affecting pathogenesis and progression of atherosclerosis but it is mainly derived from experimental models and cell lines. Our goal was to investigate DNA methylation in human atherosclerotic plaques starting with the methylation status of CpG islands in the promoter of *DAPK* gene which is involved in apoptosis and therefore plays a critical role in the remodeling of the plaque.

Patients and methods: Fifteen patients with vascular occlusive disease had their carotid atheromatous plaque surgically removed (>70% stenosis, clear evidence of atherosclerosis in the patients, all with abnormal blood lipid profile). After obtaining informed consent, we thawed part of the frozen carotid tissue and DNA was extracted (Macherey-Nagel, Germany). It was then subjected to sodium-bisulfite conversion (Zymo, USA). Methylation status was examined with methylation-specific PCR (MSP) by using gene specific primers for the promoter of *DAPK* gene (IDT, USA).

Results: According to our results, we did not detect methylation in the promoter of the *DAPK* gene. All converted samples tested positive for unmethylated sequence. Negative and positive controls for both fully methylated and unmethylated MSP reactions were as expected.

Conclusions: In our pilot study, no DNA methylation of *DAPK* gene promoter was detected however we plan to expand our sample size and examine also other genes that are critical for smooth muscle cell migration and mitotic switch.

Poster Session

MONOCYTIC NOX4, A NOVEL SOURCE OF INTRACELLULAR ROS, LOCALIZES TO REDOX SIGNALING COMPLEXES AND IS REQUIRED FOR OXLDL-INDUCED MACROPHAGE DEATH

C.F. Lee[1], **Reto Asmis**[1,2]

[1]Department of Biochemistry, [2]Department of Clinical Laboratory Sciences, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Cell death of human monocyte-derived macrophages (HMDM) induced by OxLDL is caspase-independent and requires the activation of two independent pathways: depletion of intracellular glutathione and collapse of the glutathione redox state and the increased formation of intracellular ROS. The source of these ROS, however, was not known. We now identified a new member of the Nox family, Nox4, in human monocytes and HMDM. OxLDL up-regulated Nox4 mRNA expression but not Nox1, 2, 3 or 5 mRNA. Using a novel monoclonal antibody directed against Nox4, we showed that OxLDL concomitantly up-regulates Nox4 and $p22^{phox}$ protein levels. Confocal microscopy and immunoprecipitation studies showed that Nox4 co-localize with $p22^{phox}$, suggesting that an active Nox4/$p22^{phox}$ complex is present in HMDM. Nox4 also colocalizes with components of the active focal adhesion complex, indicating that under physiological conditions Nox4 may play a role in macrophage adhesion and migration. Inhibition of MEK but not p38-MAPK, JNK or Jak2 prevented the up-regulation of Nox4 induced by OxLDL. Inhibition of MEK also protected against OxLDL-induced increases in ROS formation and OxLDL-mediated macrophage death. In contrast, inhibitors of p38-MAPK or JNK did not block OxLDL-induced ROS formation, and showed no protection against OxLDL. Adenovirus-delivered siRNA directed against Nox4 suppressed OxLDL-induced ROS formation and macrophage death while Nox4 overexpression enhanced ROS formation and accelerated macrophage death induced by OxLDL. Our data demonstrate that the Nox4/$p22^{phox}$ complex is induced in HMDM via the MEK/ERK pathway and mediates OxLDL-induced macrophage death, implicating monocytic Nox4 in the development and progression of atherosclerotic lesions.

Poster Session

VASCULAR ENDOTHELIAL GROWTH FACTOR INDUCES EXPRESSION OF POLY(ADP-RIBOSE)-POLYMERASE AND INHIBITOR OF CASPASE-ACTIVATED DEOXYRIBONUCLEASE IN ENDOTHELIAL CELLS: A NOVEL ANTI-APOPTOTIC MECHANISM

Nadia Al-Fakhri[1], M. Hörmann[1], L. Mey[1], Z. Kharip[1], K. Nemeth[1], H. Renz[1], M. Heidt[2]

[1]Department of Clinical Chemistry and Molecular Diagnostics, [2]Department of Cardiology, University Clinic Marburg, Marburg, Germany

Vascular endothelial growth factor (VEGF) exerts a potent anti-apoptotic effect on endothelial cells, however, few data exist on the underlying mechanisms. Here, we present a novel mechanism by which VEGF mediates cell survival through the regulation of poly(ADP-ribose)-polymerase (PARP) and inhibitor of caspase-activated deoxyribonuclease (ICAD).

Endothelial cells (HUVEC, huPAEC and cell lines) were incubated with VEGF 100pg/ml-1µg/ml and apoptosis was induced by integrin inhibition. PARP and ICAD production and function as well as signal transduction pathways were analyzed by Western blot, real-time RT-PCR and enzyme activity assays. Apoptosis was quantified by annexin V-flow cytometry. These experiments were corroborated by PARP and ICAD gene silencing experiments. VEGFR-2 and neuropilin-1 (NP-1) were analyzed by fluorescence microscopy and inhibitor experiments. VEGF dose-dependently induced PARP and ICAD on the protein and mRNA level. Endothelial apoptosis was induced by caspase activation resulting in PARP and ICAD degradation. Preincubation with VEGF dose-dependently reduced the sensitivity to apoptosis induction by 60-90% compared to control. VEGFR-2, that could be detected together with NP-1 on the cell surface, was involved in signal transduction together with JNK and Akt. mRNA knockdown revealed that the anti-apoptotic effect of VEGF was exerted through PARP and ICAD regulation.

PARP and ICAD are targets of the caspase cascade in apoptosis induction, their increased expression may also counteract apoptosis. VEGF inhibits endothelial apoptosis by increasing PARP and ICAD gene expression thereby reducing the effects of caspase activation. In arteriosclerosis, VEGF may promote endothelial cell survival and inhibit neointima development and progression through these mechanisms.

Poster Session

ROLE OF GROWTH-DIFFERENTIATION FACTOR 15 (GDF-15) IN A MOUSE-MODEL OF ATHEROSCLEROSIS

Gabriel Alejandro Bonaterra[1], J. Thogersen[2], S. Zügel[1], G. Bendner[2], S. Vorwald[2], J. Fey[2], U. Traut[2], J. Strelau[2], R. Kinscherf[1]

[1]Anatomy and Cell Biology, Philipps-University, Marburg, [2]Anatomy and Cell Biology III, Ruprecht-Karls University, Heidelberg, Germany

GDF-15 is expressed in macrophages after stimulation by several biological mediators. Interactions between peripheral blood mononuclear cells (PBMCs) and those within plaque are suggested to be of pathophysiological relevance for lipid-induced atherosclerosis. We therefore postulated that the expression of GDF-15 is associated with the development and progression of plaque, possibly through the regulation of apoptotic processes. To analyze the physiological functions of GDF-15, we generated GDF-15 deficient mice and crossbred them with apoE-deficient mice. After 10 weeks the offspring were feed for 12 weeks with a cholesterol-enriched western diet. Immunohistomorphometry of CD68 (macrophages) and α-actin (smooth muscle cells) were performed on cryostat cross-sections of the innominate artery (=brachiocephalic trunk). Lumen stenosis (LS), cell density (CD) and apoptosis (TUNEL) were also measured. Additionally, peritoneal macrophages were obtained from the apoE$^{-/-}$/GDF-15$^{+/+}$ and apoE$^{-/-}$/GDF-15$^{-/-}$ mice and incubated with 100µg/ml native or oxidized LDL for 24 h. The mRNA was analyzed by real-time PCR. ApoE$^{-/-}$/GDF-15$^{-/-}$ compared to apoE$^{-/-}$/GDF-15$^{+/+}$ mice showed a reduction of LS (15%), as well as in plaque a decrease of TUNEL positive-cells (10%) and an increase of the CD (35%). However, the percentage of macrophages and smooth muscle cells was similar in both groups of mice. Treatment of peritoneal macrophages of apoE$^{-/-}$/GDF-15$^{-/-}$ mice with ox-LDL, compared with treatment of native LDL, showed changes in the expression of several genes associated with apoptosis [e.g. BAD (-1.3-fold), Casp3 (-1,2-fold), CD40-antigen (+5-fold), Fas (-1,40-fold)]. Our results of in vivo and in vitro experiments show an evident relation between GDF-15, apoptosis and atherosclerotic lesion progression.

Poster Session

RESVERATROL REVERSES ENOS UNCOUPLING: IMPLICATION OF SIRT1

Huige Li[1], N. Xia[1], A. Daiber[2], A. Habermeier[1], G. Spanier[1], Q. Lu[1], M. Oelze[2], E. Closs[1], T. Münzel[2], U. Förstermann[1]

[1]Institut für Pharmakologie, [2]II. Medizinische Klinik und Poliklinik, Universitätsmedizin der Johannes Gutenberg-Universität, Mainz, Germany

Oxidative stress is involved in the pathogenesis of cardiovascular diseases. However, no reliable antioxidant therapy is currently available. SIRT1 is a NAD^+-dependent class III histone deacetylase. The effects of the SIRT1 activator resveratrol on superoxide production and gene expression were studied in atherosclerosis-prone apolipoprotein E knockout (ApoE-KO) mice as well as in human EA.hy 926 endothelial cells. The SIRT1 inhibitor sirtinol and SIRT1 siRNA were used to elucidate the role of SIRT1. Resveratrol treatment significantly reduced the levels of superoxide, 3-nitrotyrosine and malondialdehyde in the heart of ApoE-KO mice, which was associated with the upregulation of superoxide dismutase isoforms (SOD1-3), glutathione peroxidase 1 (GPx1), and catalase. In parallel, expression of NADPH oxidases NOX2 and NOX4 was reduced. Resveratrol also enhanced the expression of GTP cyclohydrolase I (GCH1), elevated the content of (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH_4) and reduced the superoxide production from uncoupled eNOS. In EA.hy 926 cells, SIRT1 inhibition with sirtinol or SIRT1 knockdown by siRNA reduced the effects of resveratrol on SOD1, SOD2, GPx1, GCH1, but not those on SOD3, catalase, or NOX4. In conclusion, resveratrol reverses eNOS uncoupling by reducing oxidative stress-mediated BH_4 oxidation and by increasing BH_4 biosynthesis. The majority of these effects are SIRT1-dependent. Thus, SIRT1 may be a promising target for the development of novel antioxidant therapy.

Poster Session

SHORT-TERM INGESTION OF PURPLE GRAPE EXTRACT IMPROVES ARTERIAL ELASTICITY INDEXES IN PATIENTS WITH POSTINFARCTION CHRONIC HEART FAILURE

Eleonora Vataman[1], N. Ciobanu[2], A. Grivenco[1], N. Ostapciuc[3]

[1]Heart Failure, [2]Functional Diagnostic, Institute of Cardiology, [3]Cardiology, Rehabilitation Centre 'Bucuria', Chisinau, Moldova

The polyphenols components of purple grapes, especially resveratrol are powerful antioxidants that induce molecular mechanisms leading to decreased arterial damage, increased nitric oxide, decreased platelet aggregation and endothelium-dependent vasodilatation. This study assessed the effects of ingesting purple grape extract on arterial elasticity and peripheral resistance in post myocardial infarction patients.

Methods: Thirty-five patients with documented old Q-myocardial infarction and chronic heart failure NYHA class II-III, stages C and D, mean age 56,7±2,5 years were divided into 2 groups: gr. 1 - patients with LVEF < 35% and gr. 2 - patients with LVEF ≥ 35%. Arterial elasticity indexes were analyzed using HDI/Pulse wave CR 2000 methodic at the time of enrolment and after 14 days of ingestion of standard dose of purple grape extract. All the patients were on controlled diet and preventive neurohormonal medication.

Results: After 14 days of treatment dynamic changes of the hemodynamic and arterial elasticity parameters were different in these two groups. Stroke volume index, small artery elasticity index significantly decreased in group 1 (LVEF< 35%) whereas systemic vascular resistance increased. In group 2 stroke volume index and large artery elasticity improvement was associated with systemic vascular resistance reduction.

Conclusions: Heart failure worsening induces progressive impairment of peripheral vascular function. In patients with CHF and severe LV systolic dysfunction peripheral artery elasticity was worsening, indicating irreversible remodeling.

Poster Session

PENTRAXIN 3 (PTX3), A NOVEL CARDIOVASCULAR BIOMARKER, IS EXPRESSED IN VASCULAR SPECIMENS OF PATIENTS WITH CORONARY ARTERY DISEASE (CAD)

Ivana Hollan[1,2], M. Nebuloni[3], B. Bottazzi[4], K. Mikkelsen[5], Ø. Førre[6], P.L. Meroni[4], Feiring Heart Biopsy Study Group

[1]Revmatismesykehuset, Lillehammer, [2]Feiring Heart Clinic, Feiring, Norway, [3]Department of Pathology, [4]University of Milan, Milan, Italy, [5]Dep. of Rheumatology, Hospital for Rheumatic Diseases, Lillehammer, [6]University of Oslo, Oslo, Norway

Background: PTX3, which belongs to the same protein family as C-reactive protein (CRP), plays an important role in innate immunity. Compared to CRP, the PTX3 response is faster, and PTX3 seems to be a better marker of local inflammation in CV system and of CV disease severity.

Aims: To look for presence of PTX3 in the aorta of patients with coronary artery disease (CAD) with and without RA co-morbidity.

Methods: Specimens from ascendent aorta (containing aortic adventitia with epicardium) routinely removed during CABG surgery in 19 RA and 20 non-RA patients were examined by histology/immunohistochemistry for presence of inflammatory cell infiltrates (ICIs), fibrosis and PTX3. The findings were semiquantified.

Results: The groups had similar age and sex distribution. PTX3 was expressed in all aortic specimens, in connection to adventitial and/or epicardial ICIs and/or fibrosis. The inflammatory changes were localized around vasa vasorum and/or diffusely throughout the tissue. Adventitial ICIs, containing CD4+ and CD20+ cells, were observed in 79% RA and 65% non RA patients, and epicardial ICIs in 84% RA and 85% non-RA patients. No significant associations between ICIs, PTX3 and fibrosis and RA co-morbidity and smoking were observed (which might be due to small sample size). The extent of PTX3 depositions correlated to the extent of ICIs (r^2=0.43, p=0.007).

Conclusion: Inflammation in deep vascular layers of the aorta of CAD patients occurred frequently, and involved PTX3. This inflammation may contribute to atherogenesis, e.g. via vasa vasorum impairment. PTX3 might be a useful biomarker of vascular inflammation.

Poster Session

DIRECT INVESTIGATION OF BIVALENT MANGANESE IN ATHEROSCLEROTIC PLAQUE BY MEANS OF HIGH-FREQUENCY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

Albert Galyavich[1], G. Mamin[2], E. Kovaleva[2], S. Orlinskii[2], V. Putliaev[2], M. Salakhov[2], N. Silkin[2], R. Khairullin[3], Y. Chelyshev[1]

[1]Kazan State Medical University, [2]Kazan State University, [3]Interregional Clinical-Diagnostics Center, Kazan, Russia

Objectives: High-frequency electron spin resonance - electron-nuclear double resonance (ESR-ENDOR) spectroscopy allow identification of complexes off transition metals.

Materials and methods: 21 samples from aorta walls from men aged from 40 to 60 years, affected by atherosclerosis, were studied. Samples have been classified by the degree of their calcification. For the analysis of the obtained results samples of nanocrystalline hydroxyapatite doped by manganese ions (Mn^{2+}) were used. EPR spectra were investigated on the Bruker Elexsys 680 spectrometer, frequency of 93.5 and 9.5 GHz in temperature range 4.2-300K. Continuous wave and pulse modes of registration were used, with the use of two- and three-pulse sequences to detect the electronic spin echo. ENDOR spectra were measured by means of Mims pulse sequence.

Results: Mn^{2+} ions concentration correlated with the intensity of calcification. As the intensity of calcification increased, the concentration of Mn^{2+} centers decreased. Spectroscopic ESR and ENDOR characteristics of detected paramagnetic Mn^{2+} centers were different from the Mn^{2+} centers' characteristics in nanocrystalline hydroxyapatite. This points on that the detected paramagnetic centers are located in the organic constituent of the tissue matrix vessel wall. This localization is distinct from localization of carbonate ion in an atherosclerotic plaque. Presumably the ESR spectrum of Mn^{2+} ions in atherosclerotic plaque is caused by the Mn-superoxide dismutase localised in mitochondria.

Conclusion: ESR spectroscopy has allowed to detect the presence of Mn^{2+} complexes in tissue matrix vessel walls and to reveal the correlation between concentration of such complexes and the atherosclerotic process intensity.

Poster Session

THE IMPACT OF CIGARETTE SMOKE ON THE ADHESION PROPERTIES OF HUMAN MONOCYTES TO ENDOTHELIAL CELLS IN VITRO

Michael Lietz[1], C. Berger[1], H.-P. Lammerich[1], Y. Steffen[1], K. Stolle[1], S. Lebrun[2], J. Schueller[1], T. Wallerath[1]

[1]Philip Morris Research Laboratories GmbH, Cologne, Germany, [2]Philip Morris International R&D, Neuchatel, Switzerland

Cigarette smoking is a well-known risk factor for the development and progression of atherosclerosis. However, the mechanisms involved are not well understood. One early step in the initiation of atherosclerosis is the adhesion of monocytes to the vascular endothelium. The objective of this project was to investigate the impact of cigarette smoke (CS) on the adhesion properties of human monocytic cell line Mono Mac 6 (MM6) to human umbilical vein endothelial cells (HUVECs) in vitro.

CS was bubbled through phosphate-buffered saline (PBS) and further diluted with cell culture medium to concentrations of 0.045 or 0.09 puffs/ml. HUVECs were exposed to CS preparations for 2 or 4 hours, and a gene array analysis (Affymetrix) was performed. Up-regulation of three different adhesion molecules-vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial selectin (E-Selectin)-was confirmed by real-time polymerase chain reaction (PCR) and InCell-Western.

To test whether the CS-induced up-regulation of adhesion molecules on the surface of the HUVECs was sufficient for the adhesion of MM6 cells, an in-vitro adhesion assay under static conditions was established. Additional adhesion experiments with blocking antibodies against VCAM1, ICAM1, and E-Selectin showed E-Selectin to have the strongest impact on the CS-induced adhesion of MM6 cells to HUVECs. Adhesion of peripheral blood mononuclear cells is reported to be higher in smokers than non-smokers; therefore, the adhesion properties of CS-exposed MM6 cells to untreated HUVECs were also investigated. No increased adhesion was detected.

Conclusion: Exposure to CS increases the adhesion of human monocytes to HUVECs in vitro.

Poster Session

RAPID INCREASE OF THE NOS INHIBITOR ASYMMETRIC DIMETHYLARGININE (ADMA) IN ERYTHROCYTE LYSATES DUE TO PROTEOLYSIS OF ADMA-CONTAINING PROTEINS

Mariska Davids[1,2], A. van Hell[1], T. Teerlink[1,2]

[1]Department of Clinical Chemistry, [2]Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, Amsterdam, The Netherlands

Introduction: Elevated plasma levels of the endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA) are associated with cardiovascular disease. Generation of ADMA and its degradation by dimethylarginine dimethylaminohydrolase (DDAH) are intracellular events. Consequently, ADMA in plasma is often regarded as cellular spill over. Recent data suggest that hemolysis-induced release of ADMA may contribute to plasma levels. To clarify the mechanism responsible for this release, the origin of ADMA released from erythrocytes upon in vitro lysis was investigated.

Methods: Lysed erythrocytes were incubated at 4°C or 37°C for 18h in the absence or presence of the proteasome inhibitor Nα-Tosyl-Lys-chloromethylketon, and/or a protease inhibitor cocktail. Intact erythrocytes served as controls. Free and total ADMA (free plus protein-incorporated ADMA after acid hydrolysis) were measured with HPLC.

Results: After lysis followed by incubation at 37°C, free ADMA levels increased from 0.9 ± 0.02 µmol/L at t=0h to 7.8 ± 0.06 µmol/L at t=18h, equivalent to 4% and 32% of the total ADMA content. This increase was much smaller at 4°C and negligible upon incubation of intact erythrocytes. Inhibition of the proteasome and proteases decreased generation of free ADMA at 37°C, especially when combined. Total ADMA in lysed erythrocytes was 24.7 ± 2.74 µmol/L and did not change upon incubation, indicating absence of *de novo* protein methylation and DDAH activity.

Conclusion: Generation of free ADMA upon erythrocyte lysis is caused by proteasomal and proteolytic degradation of methylated proteins. This may contribute to the elevation of ADMA levels in plasma observed in hemolysis-associated diseases.

Poster Session

THE AROMATASE GENE EXPRESSION IN STROMAL VASCULAR FRACTION CELLS (SVF) OF HUMAN SUBCUTANEOUS VERSUS ABDOMINAL ADIPOSE TISSUE

Aldona Dembinska-Kiec[1], B. Kiec-Wilk[1], B. Nowak- Zapala[1], W. Dudek[2], U. Czech[1], U. Razny[1]

[1]Department of Clinical Biochemistry, Jagiellonian University Collegium Medicum, [2]Obstetrician Department, Regional Specialist Hospital, Cracow, Poland

Introduction: Oestrogenes protective for cardiovascular system may also promote certain cancer development. Obesity is connected with risk for CAD as well as for cancer. Abdominal adipose tissue is more active as the source of adipokines.. Aromatase the important enzyme of oestrogen synthesis also expressed in adipose tissue. Dietary free fatty acids (FFA) and its metabolites modify differentiation of the SVF preadipocytes , thus its adipogenic properties.

The aim of this study was to investigate influence of dietary FFA on the *aromatase* gene expression in the human SVF of both types of adipose tissue (AT) depot.

Material and methods: Subcutaneous AT was excited during surgical procedure and SVF cells were isolated and incubated with 10-30µM of arachidonic (AA), palmitic (PA), eicosapentaenoic (EPA) or oleic acid (OA) in SVF-Adipo as well as in endothelial cell (SVF-Angio) differentiation promoting media. The aromatase gene expression was estimated using real-time PCR and *GAPDH as* housekeeping gene.

Results: In non-differentiated SVF, OA significantly up-regulated while AA and EPA down-regulated aromatase expression. There was no significant changes in aromatase expression in SVF during differentiation into adipocytes. In SVF-Angio PA and OA down-regulated while AA and EPA up-regulated expression of the analyzed gene.

Conclusion: Obesity and ageing (postmenopausal period) increases expresion of aromatase gene in the abdominal AT. In premenopausal young women the low BMI predisposed to the higher aromatase expression in the subcutaneous AT. AA and EPA increase aromatase gene expression in preangioblasts, when decrease in preadipocites.

Poster Session

CHLOROGENIC ACID ATTENUATES ADHESION MOLECULES UPREGULATION IN IL-1B- TREATED HUVECS

Weng-Cheng Chang[1], C.-H. Chen[2], Y.-M. Yu[3]

[1]Graduate Institute of Medical Sciences, Chang Jung Christian University, Tainan, [2]Nutrition, China Medical University, Taichung, [3]Nutrition and Health Sciences, Chang Jung Christian University, Tainan, Taiwan R.O.C.

Introduction: Expression of cell adhesion molecules (CAM) on the endothelium and the attachment of monocytes to endothelium may play a major role in the early atherogenic process. Chlorogenic acid is a phenolic compound present in coffee, apples, pears, berries, almonds, artichokes and aubergines. Previous studies have indicated that chlorogenic acid possesses antioxidant activity in vitro.

Aims: We investigated the effects of chlorogenic acid and probucol on monocyte-like adhesion, adhesion molecule expression, NF-kB translocation and ROS production in IL-1β-induced human umbilical vein endothelial cells(HUVECs).

Results: Chlorogenic acid dose-dependently suppressed IL-1β-induced mRNA expression of vascular cell adhesion molecule-1, intercellular cell adhesion molecule-1 and endothelial cell selectin. Chlorogenic acid also suppressed the IL-1β-induced production of ROS. We also observed that chlorogenic acid attenuated or blocked IL-1β-induced nuclear translocation of nuclear factor-κB subunits p50 and p65, which in turn attenuated CAM expression at the transcription level. Furthermore, chlorogenic acid significantly reduced the adhesion of human monocyte cells (U937) to IL-1β-treated HUVECs in a dose-response manner.

Conclusions: We conclude that chlorogenic acid exhibit anti-inflammatory effects in HUVECs by inhibition of U937 monocyte-like adhesion, adhesion molecule expression, NF-kB translocation and ROS production. The anti-inflammatory activity of chlorogenic acid in HUVECs suggests that chlorogenic acid could be useful in the prevention of atherosclerosis.

Poster Session

CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) PREVENTS VASCULAR SMOOTH MUSCLE CELL CALCIFICATION *IN VITRO* AND *IN VIVO*

Wei Kong[1], Y. Du[1], Y. Wang[2], L. Wang[1], C. Tang[1], X. Wang[1]

[1]Department of Physiology and Pathophysiology, Peking Universtiy Health Science Center, [2]Department of Nephrology, Peking University Third Hospital, Beijing, China

Vascular calcification is prevalent in end-stage renal disease, atherosclerosis, diabetes and aging, and is a major risk factor for cardiovascular morbidity and mortality. Once considered a passive process of calcium and phosphate precipitation, it has emerged as an actively regulated form resembling cartilage and bone formation. Cartilage oligomeric matrix protein (COMP) is an extracellular glycoprotein expressed abundantly in cartilage and plays a key role in chondrogenesis. Our recent data indicates COMP may also play important role on maintaining homeostasis of vascular smooth muscle cells (VSMCs). The aim of the current study therefore is to elucidate the potential effect of COMP on VSMCs/vascular calcification. COMP protein level was markedly reduced in mineralized VSMC induced by β-glycerophosphate, as well as in calcified arteries of 5/6 nephrectomized rats fed with high-phosphate diet. Similarly, COMP protein level was significantly decreased in calcium chloride triggered calcified VSMCs or rat abdominal aorta. In accordance, ectopic expression of COMP greatly rescued high concentration of calcium or phosphate initiated calcification of VSMCs in vitro, and of rat abdominal artery in vivo. In contrast, silencing of COMP remarkable promoted VSMCs calcification. Concurrently, COMP overexpression significantly inhibited whereas COMP knockdown facilitated osteogenic marker genes Runx2/Cbfa 1, Sox9 or Msx2 expression by VSMCs, indicating COMP may act as a repressor of VSMCs osteochondrogenic transition. In summary, our data suggests COMP is a novel inhibitor of vascular calcification. Further investigations aimed at regulation of COMP expression or integrity will shed light on discovery novel therapeutic target of pathological vascular calcification.

Poster Session

MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IS A BIOLOGICAL MARKER FOR VASCULAR INFLAMMATION INDUCED BY ENVIRONMENTAL TOXICANTS

Bernhard Hennig, Z. Majkova, M. Toborek

University of Kentucky, Lexington, KY, USA

Atherosclerosis, the primary cause of heart disease and stroke, is initiated by dysfunction of the vascular endothelium, and risk factors include environmental exposure to persistent organic pollutants. We tested the hypothesis that polychlorinated biphenyls (PCBs) can induce endothelial expression of MCP-1, a chemokine that attracts monocytes into sub-endothelial space in the early stages of atherosclerosis. Exposure to coplanar PCBs 77 and 126, as well as 2,3,7,8-tetrachlorodibenzo-p-dioxin, increased MCP-1 expression in primary endothelial cells. MCP-1 up-regulation by PCB77 was prevented by the aryl hydrocarbon receptor (AhR) antagonist a-naphthoflavone, as well as the antioxidant N-acetyl cysteine. MCP-1 up-regulation by PCB77 was also blocked by pharmacological inhibitors of p38 and c-Jun N-terminal kinase (JNK), but not ERK1/2. Caveolae are membrane microdomains involved in regulation of pro-inflammatory signaling pathways in vascular endothelial cells. We provide evidence that intact caveolae are required for MCP-1 induction by PCB77, and that silencing of caveolin-1 using siRNA can abolish MCP-1 up-regulation. Furthermore, treatment with PCB77 induced aortic mRNA expression and plasma protein levels of MCP-1 in control, but not caveolin-1-deficient mice. In conclusion, these results demonstrate that coplanar PCBs induce MCP-1 expression by endothelial cells and that this effect is mediated by functional caveolae and AhR, as well as by p38 and JNK MAPK signaling pathways. Our data further support a key role for MCP-1 as a biological marker for vascular inflammation induced by persistent organic pollutants. (Supported by grants from NIEHS, NIH (P42ES07380) and UK AES)

Poster Session

EXPRESSION OF VARIOUS ISOFORMS OF GLUTATHIONE PEROXIDASE IN THE AORTA OF GPX-1-/-APOE-/- MICE DURING ATHEROSCLEROTIC LESION DEVELOPMENT

Fei Cheng[1], M. Torzewski[2], A. Degreif[1], J. Almering[1], F. Haeuser[1], H. Rossmann[1], K.J. Lackner[1]

[1]Institut für Klinische Chemie und Laboratoriumsmedizin / Universitätsmedizin Mainz, Mainz, Germany, [2]Institut für Klinische Chemie und Hämatologie des Kantons St. Gallen, St. Gallen, Switzerland

Background: We have shown previously that deficiency of glutathione peroxidase 1 (GPx-1) accelerates and modifies atherosclerotic lesion progression in apolipoprotein E-deficient (ApoE-/-) mice. Here, we studied the expression of GPx-1, GPx-2, GPx-3 and GPx-4 in the aorta of GPx-1-/-ApoE-/- and ApoE-/- control mice during atherosclerotic lesion development.

Methods and results: At 8 weeks of age, female apolipoprotein E-deficient mice with and without glutathione peroxidase-1 deficiency were placed on an atherogenic Western-type diet for another 6 and 12 weeks and expression of different GPx isoforms was investigated by in situ-hybridization. GPx-1 expression in ApoE-/- control mice was confined to macrophages, whereas smooth muscle cells showed no expression. GPx-3 as well as GPx-4 was exclusively expressed in macrophages and fatty tissue of mouse aorta. In macrophages, expression of GPx-3 was comparable in GPx-1-/-ApoE-/- and ApoE-/- control mice whereas GPx-4 expression was higher in GPx-1-/-ApoE-/- compared to ApoE-/- control mice. GPx-2 expression could be detected neither in GPx-1-/-ApoE-/- nor in ApoE-/- control mice.

Conclusions: GPx-1 is almost exclusively expressed in macrophages from atherosclerotic lesions of ApoE-/- mice, while GPx-3 and GPx-4 are expressed in both macrophages and fatty tissue of GPx-1-/-ApoE-/- and ApoE-/- mice. In GPx-1-/-ApoE-/- mice, GPx-4 expression is increased compared to ApoE-/- mice. This most likely reflects upregulation in response to the lack of GPx-1 activity and may contribute to the comparably benign phenotype of GPx-1 deficient mice.

Poster Session

ANALYSIS OF ARACHIDONIC ACID METABOLITES AFTER INFLAMMATORIC ACTIVATION OF PRIMARY HUMAN MONOCYTES BY HYBRID QTRAP MASS SPECTROMETRY

M. Brügel[1], L. Kortz[1], S. Hausschildt[2], G.M. Fiedler[1], J. Thiery[1], **Uta Ceglarek**[1]

[1]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, [2]University of Leipzig, Leipzig, Germany

Eicosanoids are lipid signaling molecules that represent central effectors of inflammation. The aim of our study was to investigate the eicosanoid response of activated primary human monocytes by combining liquid chromatography (LC) and hybrid triple quadrupole/linear ion trap mass spectrometry (QTrap MS).

Human buffy coat monocytes from healthy blood donors were isolated using density gradient centrifugation and elutriation. Incubation was performed with lipopolysaccharide (LPS), zymosan, lipoteichoic acid (LTA) and oxidized LDL (ox-LDL). The cell supernatants were purified and measured by LC-QTrap MS. The monitored 55 eicosanoids included prostaglandins (PG), thromboxans (TX), hydroxy fatty acids (HETEs), leukotrienes (LT) and epoxy fatty acids (EET).

The activation of human monocytes with LPS resulted in a release of 8 different eicosanoids, including representatives of the COX-, LOX- and CYP450-pathway. The intra-assay variability of detected eicosanoids after LPS-incubation ranged between 1.6 and 7.4%. Compared to the activation with LPS, LTA induced a weaker response of eicosanoids. The activation with zymosan resulted in a release of 12 different eicosanoids, including representatives of the COX-, LOX- and CYP450-pathway. Unlike LPS and LTA, incubation with zymosan resulted in a release of leukotriens. The activation with ox-LDL resulted in a release of 10 eicosanoids, including representatives of the COX, LOX and CYP450 pathway. The analysis of ox-LDL revealed, that isoprostanes are released even during oxidation of LDL.

We were able to show diverse eicosanoid patterns elicited by LPS, zymosan and LTA. This may reflect the different signal cascades in AA metabolism that are activated by the different stimuli.

Poster Session

MITOCHONDRIAL INDEPENDENT APOPTOSIS IS STIMULATED IN MONONUCLEAR CELLS FROM HYPERTRIGLYCERIDEMIC MICE

Helena Oliveira[1], L. Alberici[2], K. Zecchin[2], B. Paim[2], S. Mirandola[2], R. Castilho[2], A. Vercesi[2]

[1]Physiology and Biophysics, [2]Clinical Pathology, State University of Campinas, Campinas, Brazil

We have recently demonstrated that increased rates of reactive oxygen species (ROS) generation by extra-mitochondrial enzymes promote activation of the mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) in livers of hypertriglyceridemic (HTG) mice. The resulting mild uncoupling mediated by mitoK_{ATP} protects mitochondria against oxidative damage. Here we studied whether immune cells from HTG mice also present higher activity of mitoK_{ATP} and its consequences to cell redox state and apoptosis rate. The results showed that spleen mononuclear cells from HTG mice have a higher activity of the mitoK_{ATP} channel that increases cell resting respiration and decreases mitochondrial ROS release rates. However, total cell ROS and apoptosis were increased in HTG mice mononuclear cells. Apoptosis was independent on the cytochrome C release and associated with increased caspase-8 activity. Accordingly, a reduced number of blood circulating leukocytes was found in HTG mice. Inhibition of mitoK_{ATP} by selective antagonists further increased apoptosis in HTG cells. Incubation with HTG mice serum induced apoptosis in control but not in HTG mononuclear cells. These results indicate that mitoK_{ATP} activity acts as a protective mechanism partially preventing cell death induced by elevated levels of plasma triglycerides in HTG mice.

Poster Session

OSTEOPONTIN GENE VARIATION AND CARDIO/CEREBROVASCULAR DISEASE PHENOTYPES

B. Schmitz[1], **Klaus Schmidt-Petersen**[1], E. Brand[2], R. Telgmann[1], V. Nicaud[3], C. Hagedorn[1], J. Labreuche[4], C. Dördelmann[1], A. Elbaz[5], M. Gautier-Bertrand[5], J. Fischer[6], A. Evans[7], C. Morrison[8], D. Arveiler[9], M. Stoll[1], P. Amarenco[4], F. Cambien[10], M. Paul[11], S.-M. Brand-Herrmann[1]

[1]Leibniz-Institute for Arteriosclerosis Research, University of Münster, [2]Department of Internal Medicine D, Nephrology and Hypertension, University Hospital Münster, Münster, Germany, [3]INSERM, UMR S 525, Université Pierre et Marie Curie-Paris6, [4]Department of Neurology and Stroke Centre, Bichat University Hospital, [5]INSERM U708, Neuroepidemiology Hôpital de la Salpêtrière, Université Pierre et Marie Curie-Paris, Paris, France, [6]Institute for Pharmacology, University Hospital Essen, Essen, Germany, [7]MONICA Projects in UK and France, Belfast, [8]MONICA Projects in UK and France, Glasgow, UK, [9]MONICA Projects in UK and France, Strasbourg, [10]INSERM UMR S 525, Université Pierre et Marie Curie-Paris, Paris, France, [11]Faculty of Health, Medicine, and Life Science, Maastricht University, Maastricht, The Netherlands

We aimed at associating common osteopontin (OPN) gene variants with cardiovascular disease phenotypes. We scanned the OPN gene in 190 chromosomes from myocardial infarction (MI) patients and identified five variants in the promoter, three synonymous and one non-synonymous variant. All variants were investigated in case-control studies for MI (ECTIM: 990 cases, 900 controls) and brain infarction (BI) (GÉNIC: 466 cases, 444 controls). Promoter variants were functionally analyzed by bandshift assays, the coding D147D [T/C] by western blot. Allele D147D C was independently and significantly associated with lower apoB levels (P=0.044 [ECTIM] P=0.03 [GENIC]), its allele frequency was significantly lower in patients with BI compared to controls (OR [95% CI] 0.39 [0.20-0.74], P=0.004), and C allele carriers had a significantly lower frequency of presence of carotid plaques (P=0.02). Bandshifts with HepG2 and Ea.hy926 nuclear proteins did not reveal any functionality of promoter variants, whereas the OPN-441C-containing construct resulted in reduced OPN protein expression in western blots, complying with its potential protective effect on the phenotypes studied.

We here provide evidence that a portion of the OPN locus is likely to associate with cardiovascular disease-related phenotypes. However, further experiments are warranted to clarify the functional role of OPN variants.

Poster Session

INTERACTION OF PLATELET-DERIVED MICROPARTICLES WITH PLASMA LIPOPROTEINS. EFFECT ON PLATELET ACTIVATION

A. Dimitriou, J. Mitsios, **Alexandros Tselepis**

Chemistry, University of Ioannina, Ioannina, Greece

Objectives: Platelet-derived microparticles (PMPs) play an important role in atherothrombosis. We investigated the possible interaction of PMPs with plasma lipoproteins, as well as their biological role on platelet activation.

Methods: PMPs were prepared from the supernatant of washed human platelets activated with Ca^{2+}-ionophore A23187. Lipoproteins were isolated from plasma by sequential ultracentrifugation. The possible interaction of PMPs with FITC conjugated low-density lipoprotein (LDL) or high-density lipoprotein (HDL) was studied by flow cytometry. The aggregatory activity of PMPs or their complexes with LDL or HDL was studied on washed platelets.

Results: Both lipoproteins, LDL and HDL, were able to bind to PMPs in a dose-dependent manner at a lipoprotein to microparticles protein ratio ranging from 0.5 to 2.0. PMPs induced platelet aggregation in a dose-dependent manner (at a concentration ranging from 10-1.25 μg/ml) which was significantly reduced when platelets were pretreated with aspirin suggesting that it could be attributed to arachidonic acid metabolites. The binding of either LDL or HDL to PMPs significantly reduced their aggregatory effect in a dose-dependent manner.

Conclusions: This is the first study showing that both plasma lipoproteins LDL and HDL bind to platelet microparticles and significantly attenuate their aggregatory effect on human platelets. The significance of the above findings concerning the pathophysiology of atherothrombosis remains to be established.

Poster Session

EFFECT OF ACUTE INFLAMMATION ON RESPONSE OF BETA-2 ADRENOCEPTORS OF RAT KNEE JOINT VESSELS DECREASED IN PRESENCE OF DIABETES

Sohrab Hajizadeh

Tarbiat Modares University, Tehran, Iran

Introduction: Due to the vasodilatory effect of acute inflammation on blood vessels we aimed in this study to investigate the responsiveness of adrenoceptors of knee joint blood vessels of rats in presence of diabetes.

Methods: Wistar rats weighing 200-300 gr. were used in this study. Acute inflammation was induced by intra articular injection of kaolin 4% and induction of diabetes was performed by streptozotocine (55 mg/kg). Animals divided into 4 groups as: control, diabetic, inflammation and diabetic-inflammation. Laser Doppler flowmetry(LDF) technique was used for monitoring of knee joint blood flow. Vasodilatation of articular micro vessels was measured in response to topical application of different concentrations (10 -11 to 10-1) of Salbutamol.

Results: Data showed that 1- knee joint diameter and perimeter due to acute inflammation were significantly lesser in diabetic than that of inflammatory rats. 2- Responsiveness of beta-2 adrenoceptors was increased in acute inflammation. 3- Diabetes attenuated the response of beta-2 adrenoceptors in acute inflammation.

Conclusion: Based on the above mentioned results, we conclude that diabetes inhibits the vasodilatory effect of acute inflammation on knee joint blood vessels.

Poster Session

REACTIVE OXYGEN SPECIES, VIA MITOCHONDRIAL K_{ATP} CHANNELS, ACTIVATE PRO-SURVIVAL PATHWAY IN PRAVASTATIN-INDUCED CARDIOPROTECTION

Tetsunori Saikawa[1], L. Cong Thuc[1], Y. Teshima[1], N. Takahashi[1], Y. Nagano-Torigoe[1], K. Ezaki[1], K. Yufu[1], M. Nakagawa[1], M. Hara[2]

[1]Department of Laboratory Medicine and Diagnostics, [2]Department of Intenal Medicine I, Oita University, Yufu, Japan

Reactive oxygen species (ROS) are important intracellular signaling molecules and are implicated in cardioprotective pathways including ischemic preconditioning. Statins have been shown to have cardioprotective effects against ischemia/reperfusion injury, however, the precise mechanisms remain to be elucidated. We hypothesized that ROS-mediated signaling cascade may be involved in pravastatin-induced cardioprotection. Cultured rat cardiomyocytes were exposed to H_2O_2 for 30 min to induce cell injury. Pravastatin significantly suppressed H_2O_2-induced cell death evaluated by propidium iodide staining and the MTT assay. Incubation with pravastatin activated catalase, and prevented a ROS burst induced by H_2O_2, which preserved mitochondrial membrane potential. Protective effects were induced very rapidly within 10 min, which was concordant with the up-regulation of phosphorylated ERK1/2. L-NAME, 5HD, N-acetylcysteine (NAC) and staurosporine inhibited ERK1/2 phosphorylation and also reduced pravastatin-induced cardioprotection, suggesting NO, mitochondrial K_{ATP} (mitoK_{ATP}) channels, ROS and PKC should be involved in the cardioprotective signaling. We also demonstrated that pravastatin moderately up-regulated ROS generation in a 5HD-inhibitable manner. In isolated perfused rat heart experiments, pravastatin administered 10 min prior to no-flow global ischemia significantly improved left ventricular functional recovery, and also reduced infarct size, which were attenuated by the treatment with NAC. Administration of pravastatin from the beginning of reperfusion also conferred cardioprotection. Pravastatin protected the cardiomyocytes against oxidative stress by preventing the ROS burst and preserving mitochondrial function. Moderately up-regulated ROS production by mitoK_{ATP} channels opening is involved in the pro-survival signaling cascade activated by pravastatin.

Poster Session

AUGMENTED ANGIOGENESIS IN ADVENTITIA PROMOTES PLAQUE FORMATION IN ABDOMINAL AORTA OF APOLIPOPROTEIN E-DEFICIENT MICE

Kimie Tanaka[1], D. Nagata[1], Y. Hirata[1], Y. Tabata[2], R. Nagai[1], M. Sata[3]

[1]Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, [2]Biomaterials, Institute for Frontier Medical Science, Kyoto University, Kyoto, [3]Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Introduction: Exaggerated formation of vasa vasorum (VV) was detected along with atherosclerotic plaque progression. In advanced lesions, VV invades into plaques and supplies inflammatory cells into them. However, it remains unclear whether augmented VV could promote hyperlipidemia-induced plaque formation.

Methods: Slow releasing form (30 days) of basic fibroblast growth factor (bFGF) was administered focally to augment VV. bFGF (100μg/body) incorporated in acid gelatin hydrogel microspheres (AGHMs) (bFGF+AGHM, n=10), AGHMs alone (AGHM, n=7), PBS(control, n=8) was injected into periaortic areas of retroperitoneal space of 10 to 11-week-old male ApoE-deficient mice. After 2, 3, 4 and 13 weeks, the abdominal aortas were harvested with perivascular soft tissues. The adventitial micro-vessels and macrophages were visualized by staining with *Lycopersicon Esculentum* (Tomato) lectin and anti-Mac3 antibody. The same procedures were performed in age-matched wild-type mice (n=4).

Results: Larger lesions were observed only in the bFGF+AGHM group (bFGF+AGHM: $3.4x10^4 \pm 0.7 x10^4 \mu m^2$, AGHM:$0.8 x10^4 \pm 0.7x10^4 \mu m^2$, control 0 μm^2 , p=0.0002) at 13 weeks. Plaque formation was associated with augmented VV, although invasion of VV into plaques was not observed at time points. At early phase, proliferation of VV and accumulation of macrophages were observed prior to plaque formation. In wild-type mice, although bFGF induced VV proliferation, lesion formation was not observed.

Conclusions: Under hyperlipidemic conditions, adventitial administration of bFGF induces VV development, which potentially accelerates plaque formation and progression. Augmented VV might influence plaque progression not only in advanced lesion but also in initial one via inflammatory cell recruitment in adventita.

Poster Session

ATHEROPROTECTIVE EFFECTS OF SIMVASTATIN, TELMISARTAN AND RESVERATROL: INHIBITION OF MONOCYTE ADHESION TO ENDOTHELIUM EXPOSED TO NON-UNIFORM SHEAR-STRESS AND TNF-A

Katharina Urschel, I. Cicha, W.G. Daniel, C.D. Garlichs

Department of Cardiology and Angiology, University of Erlangen-Nuremberg, Erlangen, Germany

Objective: Non-uniform shear stress-induced endothelial activation promotes atherosclerotic plaque formation at arterial bifurcations via increased recruitment of inflammatory cells. Our previous study showed that non-uniform shear stress in combination with circulating TNF-α lead to increased endothelial VCAM-1 and E-selectin expression and enhanced monocyte adhesion. Based on these results, we now hypothesized that pharmacologic substances which suppress inflammation may prevent monocyte recruitment.

Methods: HUVECs seeded in bifurcating flow-through cell culture slides were exposed to shear stress overnight, followed by 2 hours stimulation with TNF-α. Subsequently, HUVECs were perfused for 1 hour with medium containing THP-1 monocytes. During flow, cells were incubated either with simvastatin - an inhibitor of HMG-CoA-reductase, telmisartan - an AT1R blocker, or resveratrol - the active compound of red wine. Adherent THP-1 monocytes were quantified by light microscopy. Endothelial adhesion molecule expression was determined by immunofluorescence.

Results: Simvastatin (1 µmol/L) suppressed non-uniform shear stress- and TNF-α-induced monocytic cell adhesion by about 30% via inhibition of VCAM-1 expression. Similar effect was observed upon treatment of endothelial cells with telmisartan: VCAM-1 expression and monocytic cell recruitment were inhibited by about 50% at concentration of 1 µmol/L. E-selectin expression was not affected by either simvastatin or telmisartan.

Resveratrol dose-dependently inhibited VCAM-1 and E-selectin expression in HUVECs exposed to non-uniform shear stress and TNF-α, and reduced monocytic cell recruitment by 50% at 20 µmol/L.

Conclusions: Anti-atherogenic pharmaceutical and natural compounds decrease TNF-α-induced recruitment of monocytic cells and the expression of adhesion molecules in endothelial cells exposed to atherogenic shear stress in vitro.

Poster Session

NEUTROPHIL IL-1R AND CHEMOKINE EXPRESSION FOLLOWING *IN VIVO* EXTRAVASATION IN PATIENTS WITH CORONARY ARTERY DISEASE

Josefin Paulsson[1], A. Moshfegh[1], E. Dadfar[1], A. Bygdén-Nylander[2], C. Held[3], S. Jacobson[1], J. Lundahl[1]

[1]Karolinska Institutet, [2]Karolinska Hospital, Stockholm, [3]Uppsala University Hospital, Uppsala, Sweden

The aim was to study neutrophil gene expression following *in vivo* extravasation in relation to the inflammatory process in patients with coronary artery disease (CAD).

In vivo extravasated neutrophils were collected by use of a skin chamber method. Neutrophils from peripheral blood and the skin chamber exudate were purified by density centrifugation. Affymetrix human Gene Chip U133 plus 2.0 and the Gene Spring GX software were used to analyse gene expression. Confirmation of gene expression was performed by RT-PCR and assessment of IL-1R signalling was studied following neutrophil *in vitro* stimulation by IL-1.

Following extravasation 400 genes were induced and 1525 genes were suppressed in neutrophils from patients with CAD compared to controls. The general induction in both CAD patients and healthy controls following neutrophil extravasation included an increased expression of IL-1R1 and NFκB together with CCL20 and CXCL2. An increased IL-1R expression on extravasated neutrophils was confirmed by flow-cytometry and immunofluorescent electron microscopy. Induction of chemokines in skin chamber exudate was confirmed by ELISA. The increased induction of chemokines following extravasation was more pronounced in CAD patients and RT-PCR confirmed a higher expression of CCL20 and CXCL2 in patients with CAD compared to healthy controls. Assessment of IL-1R signalling in neutrophils indicated induction of these chemokines following IL-1 stimulation.

The results indicate a pro-inflammatory profile with induction of CCL20 and CXCL2 in *in vivo* extravasated neutrophils that is partly mediated by IL-1, especially in patients with CAD.

Poster Session

EXPRESSION OF INFLAMMATION-RELATED GENES IN HUMAN ATHEROSCLEROTIC PLAQUES

Maria Donata Di Taranto[1], A. Morgante[2,3], U.M. Bracale[4], L. Del Guercio[4], F. Carbone[4], F.P. D'Armiento[5], M. Porcellini[4], G. Bracale[4], F. Salvatore[2,3], G. Fortunato[2,3]

[1]Fondazione IRCCS SDN, Naples, [2]CEINGE Biotecnologie Avanzate S.C.a r.l, [3]Dip. Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, [4]Dipartimento Assistenziale di Chirurgia Generale Toracica, Vascolare e Endovascolare, [5]Dipartimento di Scienze Biomorfologiche e Funzionali, Sezione Anatomia Patologica e Citopatologica, Università degli Studi di Napoli Federico II, Napoli, Italy

Aims: Atherosclerosis is an inflammatory disease which molecular mechanisms are not been completely investigated. Our aim is to perform a wide expression study of inflammation related genes in human atherosclerotic plaques.

Methods: The Human Inflammation Array (Applied Biosystems) was used to perform the mRNA quantification of 92 inflammation-related genes in 12 atherosclerotic plaques, their respective adjacent regions (with a lower grade lesion) and 7 healthy arteries. The principle of the array is the real-time PCR amplification with a TaqMan probe specific for each gene. Data analysis was performed with SDS 2.3 software with the comparative Ct method using the gene beta-2-microglobulin as housekeeping and one of analysed samples as calibrator.

Results: The mRNA levels of 42 genes result to be differently expressed: 13 genes were up-regulated in atherosclerotic plaques respect to control arteries whereas 29 were down-regulated. Their expression levels show an increasing or a decreasing trend from healthy arteries to plaque adjacent regions and to advanced plaques. These genes belong to many different functional classes. In particular, we observed the dys-regulation of genes encoding for enzymes involved in the arachidonic acid metabolism that lead to generation of prostaglandins and leukotrienes.

Conclusions: This is the first study in which the expression of a wide panel of inflammation-related genes was investigated. Results clarify the mechanisms of inflammation involvement during atherosclerotic process and highlight new possible target for anti-inflammatory therapy in cardiovascular disease.

Poster Session

LONG-TERM ENDURANCE EXERCISE ATTENUATES THE AGING PROCESS IN CIRCULATING LEUKOCYTES

Christian Werner[1], T. Fürster[1], J. Scharhag[2], M. Böhm[1], U. Laufs[1]

[1]Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg, [2]Institut für Sport- und Präventivmedizin, Universität des Saarlandes, Saarbrücken, Germany

Background: Physical activity reduces cardiovascular morbidity and mortality. Aging is the major cause of cardiovascular disease. Telomeres and telomere-associated proteins regulate aging on the cellular level. Our study examines telomere biology and senescence factors in endurance athletes and controls without physical activity.

Methods: Peripheral blood leukocytes were isolated from young track & field athletes (n=32, age 20 years, running 73 km/week), aged athletes performing regular endurance training (n=25, age 51 years, running 80 km/week, 35 years training history) and two control groups of physically inactive healthy volunteers.

Results: Telomere repeat amplification protocols revealed an activation of leukocyte telomerase in young athletes and in elderly athletes compared to controls. Western blots showed an up-regulation of the telomere-capping protein TRF2 in young as well as in aged athletes. The expression of p16 and p53 was reduced in aged athletes. Flow-FISH assays and real-time PCR measurements of leukocyte telomere length demonstrated that sedentary elder controls exhibited a significant reduction of leukocyte telomere length (FF 53%, PCR 70% vs. young controls). Importantly, there was a striking conservation of telomere length in aged athletes (FF 88%, PCR: 84% vs. young controls).

Conclusions: Beneficial effects of exercise on telomere proteins and senescence markers occur in leukocytes of young track & field athletes. In elderly athletes with a long-term history of endurance exercise we found a potent activation of leukocyte telomerase and conservation of telomere length. These findings improve the molecular understanding of beneficial vascular effects of physical activity and implicate an anti-aging effect of physical exercise.

Poster Session

CYTOPROTECTIVE AND ANTIOXIDANT PATHWAYS IN PERIPHERAL BLOOD MONONUCLEAR CELLS ARE REGULATED BY LDL CHOLESTEROL LEVELS IN FAMILIAL HYPERCHOLESTEROLEMIA

Mariarita Puntoni[1], F. Bigazzi[2], F. Sbrana[2], B. Pennato[3], T. Sampietro[2]

[1]CNR Institute of Clinical Physiology, [2]Gabriele Monasterio CNR Regione Toscana Foundation of Pisa, [3]Scuola Superiore Sant'Anna, Pisa, Italy

Background: Hypercholesterolemia and oxidative stress have been implicated as major contributors in atherosclerosis. Endothelial nitric oxide synthase (eNOS) and heme oxygenase-1 (HO-1) are cytoprotective and antioxidant pathways within the vasculature.

Methods: We evaluated whether cholesterol affects these pathways in mononuclear cells from patients blood (8 males, mean age 56±12) with Familial Hypercholesterolemia (FH), before and two days after apoB absorption by LDL apheresis (LDL-A).

Lipoprotein profile, routine blood chemistry (BC), arterial blood pressure (BP) and endothelial function monitored. The expression levels of eNOS, iNOS, HO-1 as well as the levels of phosphorylated eNOS (p-eNOS) and AKT (pAKT) in isolated mononuclear cells were determined using Western blot.

Results: Following LDL-A no changes in BP or BC were observed; mean percent reductions in CH, LDL-C, HDL-C, TG, and Lp(a) were 74%, 82%, 7%, 56%, and 86%, respectively. Two days after LDL-A CH mean dropped from 319±28 to 158±12 mg/dL, sICAM1 and sELAM1 were also significantly reduced ($p < 0.001$); mononuclear subpopulation cells varied for a CD4 10% increase and a CD8 12% decrease. The levels of eNOS (2-fold) and HO-1 (3-4-fold) as well as p-eNOS (2-3-fold) and pAKT (50-70%) were increased after cholesterol removal. In contrast, iNOS levels were decreased by 2-fold.

Conclusions: These results suggest that: *i*) increase of cytoprotective and antioxidant signals contribute to the decrease in inflammatory markers and to restoration of vascular function after cholesterol removal, *ii*) high levels of cholesterol impose an inhibition on cytoprotective/antioxidant circuits and an upregulation of inflammatory pathways, with consequently vascular dysfunction.

Poster Session

DISTINCT DEFECTS IN COLLAGEN MICRO-ARCHITECTURE UNDERLIE VESSEL WALL FAILURE IN GROWING AORTIC ABDOMINAL ANEURYSMS AND ANEURYSMS IN MARFAN SYNDROME

Jan-Willem M. Beenakker[1], J.H.N. Lindeman[2], B.A. Ashcroft[1], M. van Es[1], N.B.R. Koekkoek[1], F.A. Prins[3], J.F. Tielemans[2], H. Abdul-Hussien[3], R.A. Bank[4], T.H. Oosterkamp[1]

[1]Leiden Institute of Physics, Leiden University, [2]Dept. of Vascular Surgery, [3]Dept of Pathology, Leiden University Medical Center, Leiden, [4]Medical Biology Section, University Medical Center Groningen, Groningen, The Netherlands

An aneurysm of the aorta is a common pathology that is part of the atherosclerotic spectrum of diseases. Remarkably, while it is accepted that vessel wall weakening is caused by an impaired collagen metabolism, a clear association has only been demonstrated for the rare syndromes such as the vascular type Ehlers Danlos syndrome.

Here we show that vessel wall failure in growing aneurysms of AAA and Marfan patients is not related to a collagen defect at the molecular level. On the contrary our findings indicate similar (Marfan) or even higher collagen concentrations (AAA), and increased collagen cross-linking in the aneurysms. Using 3D-confocal imaging we show that the conditions are associated with profound defects in collagen micro-architecture. Reconstructions of normal vessel wall show that adventitial collagen fibers are organized in a loose braiding of collagen ribbons. These ribbons encage the vessel, allowing it to dilate easily, but preventing overstretching. AAA and aneurysms in Marfan syndrome show dramatically altered collagen architectures with loss of the collagen knitting. Evaluation by atomic force microscopy showed that the wall has lost its ability to stretch easily but also revealed a second defect: while vascular collagen in the normal aortic wall behaves as a coherent network, AAA and Marfan tissues do not. As a result, mechanical forces loaded on individual fibers are not distributed over the tissue.These studies demonstrate that the mechanical properties of tissue are strongly influenced by the (collagen) micro-architecture and that perturbations in the collagen networks may lead to mechanical failure.

Poster Session

THE CX3C CHEMOKINE FRACTALKINE AND GPIB ALPHA MEDIATE PLATELET TRANSLOCATION ON VON WILLEBRAND FACTOR UNDER PHYSIOLOGIC FLOW CONDITIONS

Sascha Meyer dos Santos, U. Klinkhardt, K. Scholich, K. Kuczka, S. Harder

Department of Clinical Pharmacology, University Hospital Frankfurt, Frankfurt, Germany

The membrane-anchored CX3C chemokine fractalkine (FKN) is expressed on activated endothelium and associated with the development of atherosclerosis. A flow-based adhesion assay was used to study the adhesion of platelets to immobilised FKN under physiologic flow conditions. Platelets adhered weakly to immobilised FKN (firmly adherent platelets ± SEM: 23 ± 2) at 150 s-1 wall shear rate (adhesion to control: 9 ± 2). However, platelet adhesion to vWf (716 ± 75 adherent platelets at 600 s-1) was increased in the presence of FKN (1348 ± 103). Additional platelet adhesion to FKN co-immobilised with vWf was dependent on the FKN receptor CX3CR1, pertussis toxin-sensitive signalling and activation of GPIIb/IIIa. The number of platelets rolling on vWf was likewise enhanced in the presence of FKN (175 ± 14 % of vWf-dependent translocation). The enhancement of translocation on FKN and vWf was insensitive to anti-CX3CR1 antibody, but was fully inhibited by neutralising GPIbα function. Glycocalicin, the extracellular domain of GPIbα, was covalently coupled to fluorescent microspheres and used to study the interaction of GPIbα-expressing microspheres with vWf / FKN surfaces under flow. At all tested shear rates, binding of GPIbα to FKN surfaces co-immobilised with vWf was significantly higher compared to vWf. Furthermore, glycocalicin-expressing microspheres adhered to FKN (20 ± 3 adherent microspheres at 600 s-1) significantly over non-specific background binding to albumin surfaces (7 ± 1). These data demonstrate that endothelial expressed FKN activated platelets via its cognate receptor CX3CR1, whereas platelet adhesion was predominantly mediated by GPIbα and independent of CX3CR1.

Poster Session

FIBROUS CAP THINNING AND PLAQUE VULNERABILITY AT THE PROXIMAL REGION ARE ASSOCIATED WITH INCREASED LEVELS OF CATHEPSIN L

Iwona Cicha[1], A. Wörner[1], K. Urschel[1], M. Klein[2], D. Raithel[2], W.G. Daniel[1], C.D. Garlichs[1]

[1]Department of Cardiology and Angiology, University of Erlangen-Nuremberg, Erlangen, [2]Department of Vascular Surgery, Clinic South Nuremberg, Nuremberg, Germany

Objectives: Rupture of atherosclerotic plaques is the main cause of acute coronary syndromes and ischemic strokes. We investigated the features of plaque vulnerability in relation to blood flow direction.

Methods: The occurrence of rupture, endothelial erosion, neovascularization, and hemorrhage were determined in longitudinal sections of 80 human carotid plaque specimens. Minimum thickness of the fibrous cap was measured. Cathepsin L expression was detected immunohistochemically.

Results: In 86% of ruptured plaques, rupture was observed in the upstream (proximal) region. In this region, the occurrence of intimal neovascularization and hemorrhage was increased ($P < 0.001$), whereas endothelial erosion was more frequent downstream. Strong thinning of the fibrous cap (FC) was observed upstream as compared with other plaque regions. Furthermore, immunoreactivity of the cysteine protease cathepsin L was significantly higher upstream as compared with the downstream shoulder of atherosclerotic lesions. In the ruptured plaques, thinning of the fibrous cap on the upstream side was significantly more pronounced than in the stable ones ($P < 0.001$). Rupture was also significantly associated with the presence of intraplaque hemorrhage ($P=0.041$). Interestingly, a significant increase in the numbers of cathepsin L-expressing cells was observed at the upstream shoulder of ruptured plaques, as compared with non-ruptured plaques ($P=0.044$). Moreover, cathepsin L expression was inversely correlated with minimum FC thickness upstream ($r = -0.3$, $P=0.013$).

Conclusions: The upstream region of the atherosclerotic plaque is the predominant site of rupture. Vulnerability of this region is associated with enhanced neovascularization, hemorrhage, and cap thinning induced by proteolytic enzymes.

Poster Session

ENDOTHELIAL PROGENITOR CELLS (EPCS) IN PATIENTS WITH MIXED HYPERLIPIDAEMIA

V. Chantzichristos[1], A. Agouridis[2], M. Elisaf[2], **Alexandros Tselepis**[1]

[1]Chemistry, [2]Department of Internal Medicine, University of Ioannina, Ioannina, Greece

Objectives: Circulating endothelial progenitor cells (EPCs) significantly contribute to the regeneration of endothelium. We investigated the number of EPCs in peripheral blood as well as their ability to form colonies in vitro in patients with mixed hyperlipidaemia, before and after the initiation of statin therapy.

Methods: Twelve patients (4 men, 8 women, mean age 48 years) with mixed hyperlipidaemia (LDL-cholesterol ≥160 mg/dl, Triglycerides ≥200 mg/dl) and eleven normolipidaemic apparently healthy age -and sex- matched volunteers participated in the study. Patients received rosuvastatin therapy, 40mg/day. The membrane expression of characteristic antigens of EPCs, CD34 and KDR in peripheral blood was studied by flow cytometry. Moreover, EPCs from total peripheral blood mononuclear cells were isolated and cultured on fibronectin coated 24-well culture plates with Medium 199. After 7 days of culture the number of colonies formed by EPCs was counted.

Results: EPCs in patients with mixed hyperlipidaemia exhibited reduced membrane expression of KDR compared with healthy volunteers ($p < 0.03$). Furthermore, the number of EPC colonies in patients was significantly lower compared with those of healthy subjects (47.9±39.5 versus 78.8±43.3 respectively, $p < 0.04$). Three months after rosuvastatin therapy, the expression of KDR as well as the number of colonies were significantly increased ($p < 0.05$).

Conclusions: Patients with mixed hyperlipidaemia have lower number of circulating EPCs and reduced capability of EPC colonies formation compared with apparently healthy volunteers. Rosuvastatin therapy significantly increased the expression of KDR as well as the colony forming capability of EPCs.

Poster Session

HETEROGENEITY OF HUMAN CULTURED MACROPHAGES IS GROWTH FACTOR-DEPENDENT AND AFFECTS THE EXPRESSION OF GENES RELATED TO CHOLESTEROL EFFLUX AND METABOLISM

Jani Lappalainen[1], N. Yeung[1], M. Jauhiainen[2], P.T. Kovanen[1], M. Lee-Rueckert[1]

[1]Wihuri Research Institute, [2]National Institute for Health and Welfare, Helsinki, Finland

Distinct macrophage subpopulations have been identified in the human arterial intima, with varying effects on the development of atherosclerosis. Granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) are the hematopoietic growth factors that regulate the generation and function of monocyte-derived macrophages. Here we studied cultured human macrophage gene expression profiles related to cholesterol uptake and efflux, and the cholesterol contents of macrophages differentiated under either GM- or M-CSF, and exposed to acetyl-LDL in the absence or presence of cholesterol acceptors.

We found that macrophages differentiated in the presence of M-CSF expressed higher levels of scavenger receptor SR-AI for modified-LDL. This also correlated with their higher avidity to accumulate cholesterol esters by incubation with acetyl-LDL. Expression of ABCA1, ABCG1, CD36 and SR-BI mRNA was also influenced by the two types of CSFs, suggesting that GM-CSF enables macrophages to hold greater ability for cholesterol efflux via ABCG1. Importantly, these divergent gene expression profiles, favoring a higher atherogenicity of macrophages differentiated in the presence of M-CSF, were attenuated in the foam cell phenotype; neither were they restored after release of intracellular cholesterol via different efflux pathways.

These data indicate that the "pro-atherogenic" macrophages display a protective compensatory response during cholesterol loading, at least with regards to ABCG1-mediated cholesterol efflux. This suggests that human macrophage gene profile is, besides the effect of growth factors, subjected to the presence of modified LDL and HDL particles, all of which have a global modifying effect on the intracellular cholesterol balance and foam cell formation.

Poster Session

INCREASED TIMP3 EXPRESSION IN MONOCYTE/MACROPHAGE CELLS PROTECTS FROM ATHEROSCLEROSIS IN MICE

Viviana Casagrande[1], R. Menghini[1], M. Cavalera[1], S. Menini[2], G. Pugliese[2], R. Lauro[1], M. Federici[3]

[1]University of Rome Tor Vergata, [2]University of Rome La Sapienza, [3]Internal Medicine, University of Rome Tor Vergata, Rome, Italy

Obesity and Diabetes are associated with low-grade inflammatory state which prompts to diabetes and atherosclerosis. We found that activity and expression of Tissue inhibitor of Metalloproteinase 3 (TIMP3) are decreased in muscle and atherosclerotic plaques from obese diabetic patients and mice compared to controls. Because atherosclerosis and related metabolic disorders are associated with increased macrophage accumulation in different tissues, we used a transgenic approach under control of CD68 promoter to reconstitute TIMP3 levels and we analyzed its effect on atherosclerosis progression in mice with LDLR knockout background. After 12 weeks of Western Diet LDLR-/- mice developed atherosclerotic plaques (Oil Red O staining) at aortic roots characterized by macrophage (F4/80) and T cell (CD3) infiltration. LDLR-/-TgTimp3 mice showed significant reduction of atherosclerotic plaque area and inflammatory infiltrate accompanied by increased collagen content and absence of necrotic core. Liver of LDLR-/-TgTimp3 also showed less steatosis compared with LDLR-/- littermates.

Gene expression studies performed in aortic tissue showed in LDLR-/-TgTimp3 significantly decreased expression of NOS2, NADPHox, IL1beta, TNFalpha, IL6, MCP1, CD36, CX3CL1, SCD1 and significantly increased expression of eNOS, Timp3, NRF1 and LXRalpha compared with LDLR-/- littermates.

In aorta we found decreased expression of NOS2, Socs3, IL6 and IL1beta in LDLR-/-TgTimp3 compared with LDLR-/- littermates. Immunostaining of aortic roots revealed decreased Nε-(Carboxymethyl) lysine and nitrotyrosine staining in LDLR-/-TgTimp3 compared with LDLR-/- littermates. Overall these data suggest that increased Timp3 expression from CD68 positive cells is associated to decreased inflammation in both atherosclerotic plaques and liver of mice fed a high cholesterol diet.

Poster Session

ENDOGLIN EXPRESSION IN ATHEROGENESIS IS NOT RELATED TO EITHER CHOLESTEROL LEVELS OR PLAQUE SIZE IN APOE/LDLR DOUBLE KNOCKOUT MICE

Lenka Vecerova[1], Z. Strasky[1], J. Rathouska[1], M. Slanarova[1], E. Brcakova[2], S. Micuda[2], P. Nachtigal[1]

[1]Charles University, Faculty of Pharmacy, [2]Charles University, Faculty of Medicine, Hradec Kralove, Czech Republic

Endoglin is a transmembrane glycoprotein a part of the TGF-β receptor cascade and it is known as a type III TGF-β receptor. It has been demonstrated that endoglin, SMAD proteins and eNOS play role in atherogenesis. In this study, we hypothesized whether endoglin, p-SMAD1, p-SMAD2, and p-eNOS are affected by cholesterol levels and plaque size in the mice aorta.

Female ApoE/LDLR double knockout mice were divided into 3 groups (n=24). Eight weeks old mice on chow diet ("8 weeks"), 16 weeks old mice on chow diet ("16 weeks") and mice fed eight weeks with chow and another eight weeks with cholesterol rich diet ("16 weeks chol"). Biochemical analyses of lipid profile, Oil Red area staining and western blot analysis of above mentioned proteins expression in aortas were performed. Cholesterol was significantly increased in "16 weeks chol" mice when compared with other groups. Oil red area staining increased in relation with cholesterol diet and age of mice. Endoglin expression was strongest in "16 weeks" mice when compared with other groups. However, p-SMAD1, p-SMAD2 and p-eNOS levels were not affected by either blood cholesterol levels or plaque size.

In conclusion, we suggest that neither plaque size nor blood cholesterol levels, but phenotype of the atherosclerotic plaque might be involved in the regulation of endoglin expression in the mice aorta or *vice versa*.

This work was supported by grant from The Grant Agency of Charles University in Prague number 129208/C, grant MSM 002162082 and Research project MZO 001799060.

Poster Session

LIVER-SPECIFIC IKK-B ACTIVATION SEVERELY AGGRAVATES ATHEROSCLEROSIS DEVELOPMENT IN *APOE*3-LEIDEN* MICE

Man-Chi Wong[1,2], J.A. van Diepen[1], L. Hu[3], B. Guigas[4], H.C. de Boer[5], J.A. Romijn[1,3], L.M. Havekes[1,6,7], S.E. Shoelson[8], P.J. Voshol[1], J.T. Tamsma[3], P.C.N. Rensen[1], P.S. Hiemstra[2], J.F.P. Berbée[1]

[1]Endocrinology, [2]Pulmonology, [3]General Internal Medicine, [4]Molecular Cell Biology, [5]Nephrology, [6]Cardiology, Leiden University Medical Center, [7]TNO-Biosciences, Gaubius Laboratory, Leiden, The Netherlands, [8]Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA

Introduction: The relation between hepatic inflammation and atherogenesis is poorly understood. Since NF-κB is the key regulator of hepatic inflammation, we assessed whether chronic selective activation of IKK-β in hepatocytes, which results in hepatocyte-specific enhanced NF-κB activation, aggravates atherosclerosis development in *APOE*3-Leiden* (*E3L*) mice.

Methods: *E3L* mice were crossbred with *L*iver-specific *IKK*-β (*LIKK*)-transgenic mice to generate *E3L.LIKK* and control *E3L* littermates. Mice were fed a Western-type diet (containing 0.25% cholesterol) and blood was collected every 4 weeks. Atherosclerosis was assessed after 24 weeks of diet in the aortic root.

Results: *E3L.LIKK* mice had transiently increased plasma levels of total cholesterol (TC), but only at week 8 (+43%; $P< 0.001$) and 12 (+22%; $P< 0.01$), which was confined to (V)LDL, and resulted in an enhanced cumulative TC exposure (+16%; $P< 0.05$). Expression of LIKK did not affect plasma levels of inflammatory parameters (e.g. SAA, IL-6) or blood count of proinflammatory Ly6C^{hi}-monocytes. Importantly, *E3L.LIKK* mice showed highly increased atherosclerotic lesion area (+131%; $P< 0.05$) and more advanced atherosclerosis, as manifested by a reduced number of lesion-free segments (11 vs 44%; $P< 0.01$) and an increased number of severe lesions (25 vs 14%; $P< 0.05$). This was accompanied with an increased macrophage (+76%; $P< 0.05$), smooth muscle cell (+159%; $P< 0.01$) and collagen area (+147%; $P< 0.05$).

Conclusion: Continuous selective activation of hepatic IKK-β severely aggravates atherosclerosis development in *E3L* mice. Since this finding can only partially be explained by the transient increase in plasma TC levels, we are currently investigating alternative mechanisms.

Poster Session

TWO-PHOTON MICROSCOPIC IMAGING OF PLAQUE-ASSOCIATED NEO-VASCULATURE IN MICE

Timo Rademakers[1,2], K. Douma[3], T.M. Hackeng[4], E.A.L. Biessen[1], M.J. Post[5], S. Heeneman[1], M.A.M.J. van Zandvoort[2]

[1]Pathology, [2]Biomedical Engineering, [3]Radiology, [4]Biochemistry, [5]Physiology, Maastricht University, Maastricht, The Netherlands

Neovascularization of human atherosclerotic plaques has been implicated in plaque progression, destabilization and rupture. The actual presence of neovessels in mouse plaques is controversial, partly due to inadequate detection tools, thereby hampering future study of functional implications of neovascularisation. To detect plaque-associated neovessels, vascular endothelium of aged ApoE$^{-/-}$ mice (>40 wks; chow diet) was stained *in vivo* with fluorescently labelled anti-CD31, anti-cNGR (angiogenic endothelium) or anti-CD105 (activated endothelium). Carotid arteries and abdominal aorta were imaged *in vivo*, *in situ* and *ex vivo* using two-photon laser scanning microscopy (TPLSM). Afterwards, tissues were frozen, sectioned, and stained immunohistochemically (IHC). ApoE$^{-/-}$ mice (< 16 wks) with no or small, early plaques served as controls. Advanced fibroatheromatous plaques had developed in all aged mice. Using TPLSM, perfused perivascular and intraplaque vessels could be detected in most examined vessel segments (7/8 ApoE$^{-/-}$ vs. 0/6 control, P=0.0047; *ex vivo*). TPLSM penetration depth was approximately 120-170µm, depending on plaque composition, and allowed visualization of vascular network structures undetectable by conventional histological techniques. Vessel diameter was assessed for both adventitial (two types of adventitial vasculature, median: 6.0 and 22.0µm respectively) and intraplaque (median: 6.65µm) vasculature. Presence of luminal endothelium and plaque-associated vasculature could be confirmed by IHC, albeit that resolution was considerably lower than for TPLSM. Plaque-associated vasculature was not present in controls. This study clearly shows that neovascularization does occur during plaque development in ApoE$^{-/-}$ mice. Additionally, TPLSM detection of neovessels provides high resolution structural information and facilitates extended study into origin and function of neovascularization *in vivo*.

Poster Session

DEFICIENCY OF INTERLEUKIN-1 RECEPTOR TYPE I IN NONE BONE MARROW-DERIVED CELLS INHIBITS ATHEROSCLEROSIS IN APOE-KNOCKOUT MICE

Shay Shemesh[1,2], Y. Kamari[1,3], A. Shaish[1], S. Olteanu[1], N. Relvy[1], H. Levkovitz[1], O. Fingrut[1], S. Dotan[4], M. White[4], E. Voronov[4], R. Apte[4], D. Harats[1,3]

[1]The Bert W. Strassburger Lipid Center, Sheba Medical Center, [2]The Mina and Everard Goodman Faculty of Life Science, Bar Ilan, Ramat Gan, [3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, [4]Department of Microbiology and Immunology, Ben Gurion University, Beer Sheva, Israel

Introduction: Atherosclerosis is considered a low-grade inflammatory disease in which proinflammatory cytokines are key mediators.

Interleukin (IL)-1 is instrumental in the propagation of vessel wall inflammation owing to its proinflammatory effects on endothelial cells, smooth muscle cells and macrophages, mainly through binding to the type 1 IL-1 receptor (IL-1RI).

Objective: We studied the effect of whole body or bone marrow-specific deficiency of IL-1RI by generating IL-1RI deficient mice on the background of apoE-/- (DKO) and by using the bone marrow transplantation technique.

Results: Whole body deficiency of IL-1RI inhibited atherosclerosis development in apoE-/- (20%-47% reduction in aortic sinus lesion area in three different experiments), despite similar plasma non-HDL cholesterol and an obese phenotype of DKO mice. DKO mice had a more stable plaque phenotype manifested by smaller necrotic core area and reduced macrophage content. Therefore, we studied the effect of macrophage IL-1RI deficiency on cytokine profile after activation *in vitro*. IL-1RI deficient macrophages secreted less IL-6, IL-1β and TNFα in response to oxidized LDL activation.

The bone marrow transplantation studies have shown that IL-1RI-mediated signaling in cells other than bone marrow-derived is more important for atherogenesis: DKO mice transplanted with IL-1RI-expressing bone marrow-derived cells had reduced aortic sinus lesion compared to apoE-/- mice (48% reduction) while specific deficiency of IL-1RI in bone marrow-derived cells did not reduce atherosclerosis in apoE-/- mice.

Conclusion: our studies suggest that IL-1RI in vascular wall resident and not bone marrow-derived cells plays an important role in atherogenesis and in determining plaque stability.

Poster Session

GENOMIC DIFFERENCES BETWEEN MOUSE FOAMY AND NON-FOAMY MACROPHAGES

A. Thomas[1], W. Eijgelaar[2], M. Daemen[2], **Andrew Newby**[1]

[1]Bristol Heart Institute, University of Bristol, Bristol, UK, [2]Department of Pathology, University of Maastricht, Maastricht, The Netherlands

We established a method for harvesting large numbers of foamy and non-foamy macrophages from mice, in order to compare their transcriptomes.

Macrophages were isolated from subcutaneous sponges from C57/Bl6 (normal diet: NFM) or ApoE mice (high-cholesterol diet: FCM) and purified based on their buoyant density and/or by differential plating. RNA was isolated from the cells, and FCM and NFM compared using Illumina BeadChips. Differentially expressed genes underwent Ingenuity Pathway Analysis as well as GO annotation and clustering using DAVID Bioinformatics Resources, with subsequent RT-PCR validation.

Each mouse yielded 4.6±2.6 $x10^6$ macrophages (92±8.6% pure). Pathway analysis indicated that several activation, signalling and biosynthesis pathways were different, which was confirmed using RT-PCR. Furthermore FCM overexpressed pro-thrombotic α2-antiplasmin-1 (↑4.8-fold), fIIr (↑2.2-fold), fVII (↑1.7-fold), fXIII (1.7-fold), and underexpressed fibrinolytic genes uPAr (↓1.9-fold) and uPA (↓1.3-fold). The FCM also overexpressed cathepsins C, E and H (↑2-20-fold) and several extracellular matrix proteins (including collagens type I, V, VI (↑2-6-fold), decorin (↑4.7-fold) and biglycan (↑5.8-fold)).

A mouse model that yields high numbers of pure FCM and NFM has been characterised, and the genome of the macrophages compared. Elevated levels of thrombotic genes were found in FCM. This, combined with changes in matrix production and matrix proteases, may explain how atherosclerotic plaques with high levels of FCM are vulnerable to thrombotic events.

Poster Session

PLEIOTROPIC EFFECTS OF ATORVASTATIN AND TGF-B/ENDOGLIN CASCADE IN THE VESSEL WALL OF APOE/LDLR DOUBLE KNOCKOUT MICE

Zbynek Strasky[1], L. Vecerova[1], M. Slanarova[1], J. Rathouska[1], E. Brcakova[1], S. Micuda[2], P. Nachtigal[1]

[1]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Biological and Medical Sciences,
[2]Charles University in Prague, Faculty of Medicine, Department of Pharmacology, Hradec Kralove, Czech Republic

Endoglin is a transmembrane glycoprotein a part of the TGF-β receptor cascade and it is known as a type III TGF-β receptor. In our recent paper we demonstrated that administration of cholesterol-rich diet with atorvastatin (ATV) decrease cholesterol levels and increase the expression of endoglin/SMAD2/eNOS in the aorta. In this study, we studied the pleiotropic effects of atorvastatin on the expression of type II TGF-β receptor, endoglin, p-SMAD2, p-SMAD1 and p-eNOS in the mice aorta.

Two-month-old female ApoE/LDLR double knockout mice were divided into two groups. The control group (n=8) was fed with the chow diet whereas in the ATV group (n=8), atorvastatin at dose 50 mg/kg per day was added to the same diet. Biochemical analysis of lipid profile, immunohistochemical and western blot analysis of above mentioned protein expressions in aortas were performed. Administration of atorvastatin resulted in a significant increase in total cholesterol and VLDL cholesterol when compared with control mice. On the contrary, the area of atherosclerotic lesion was decreased after ATV treatment. Western blot analysis revealed induced TGF-β IIR, endoglin, p-SMAD2, p-SMAD1 and p-eNOS expression in ATV treated mice when compared with control mice.

In this study, we demonstrated that atorvastatin increase endoglin, p-SMADs and p-eNOS expressions in the vessel wall of ApoE/LDLR double knockout mice beyond its lipid lowering effects suggesting potential protective role of endoglin cascade in atherogenesis.

This work was supported by grant from The Grant Agency of Charles University in Prague number 129208/C, grant MSM 002162082 and Research project MZO 001799060.

Poster Session

OXIDIZED-HEMOGLOBIN INDUCES INNATE IMMUNE CELL CHEMOTAXIS, MONOCYTE ARREST AND THEIR SUBINTIMAL MIGRATION INTO EARLY ATHEROSCLEROTIC TISSUE

Brigitta Buttari[1], E. Profumo[1], G. Domenici[1], M. Leopizzi[2], C. Di Cristofano[2], R. Businaro[3], G. Di Giammarco[4], R. Riganò[1]

[1]Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Rome, [2]Medicina Sperimentale, [3]Anatomia Umana, Università di Roma, Sapienza, Roma, [4]Divisione di Cardiochirurgia, Policlinico SS Annunziata, Università di Chieti, G. d'Annunzio, Chieti, Italy

Background and aims: Signals that attract leukocytes to atherosclerotic plaque are partly defined. Our findings indicate that human hemoglobin (Hb) acts as a "self" antigen in patients with carotid atherosclerotic plaques. Aims of the study were to explore the ability of oxidized-Hb to act as a chemoattractant for innate immune cells and its possible role in atherogenic cell recruitment.

Methods: Chemotaxis of monocytes and dendritic cells in response to Hb was studied in vitro using Transwells inserts and in vivo by establishing a mouse model of acute peritonitis. The ability of monocytes to adhere on endothelial layer (EA.hy 926) exposed for 2 hours to oxidized Hb was studied in static and flow dynamic conditions using a parallel wall flow chamber system mounted on inverted microscope (shear stress 2 dynes/cm2 for 2 min). Aortic tissue from patients with thoracic aortic aneurysm was perfused with 10 µg/ml of Hb over 4 hours and then with fluorescence-labelled monocytes. After 2 hours, non-adherent cells were washed away and fixed aortic tissue sections were analyzed by fluorescence microscope.

Results: oxidized-Hb acts in vitro and in vivo as a chemoattractant for innate immune cells. The short-term incubation of endothelial monolayer with oxidized-Hb results in a 2-fold increase of firm shear-resistant monocyte adhesion under flow conditions. Exposure of aortic tissues to oxidized-Hb triggered monocyte recruitment in early atherosclerotic aorta.

Conclusion: Oxidized-Hb contributes to innate immune cell infiltration, monocyte arrest and their subintimal migration into early atherosclerotic tissues with consequent amplification of the inflammatory response.

Poster Session

EXPRESSION OF GENES AND PROTEINS IN ASCENDING THORACIC AORTIC ANEURYSMS AND SMOOTH MUSCLE CELLS ISOLATED FROM ANEURYSMAL SAMPLES

Zelal Adıgüzel[1], M. Serhatlı[1], O. Kaçar[1], A. Tuncer[2], E. Tuncer[2], K. Baysal[1]

[1]Molecular Cell Biology, TUBITAK MRC Genetic Engineering and Biotechnology Institute, Kocaeli, [2]T.C. Ministry of Health, Kartal Koşuyolu, Advanced Training and Research Hospital, Istanbul, Turkey

Aortic aneurysms are formed by an irreversible weakening of a certain part of the aorta, leading to localized dilatation of the vessel. The alterations in the vessel wall leading to this weakening are reported as; medial degeneration, increased expression of matrix metalloproteinases (MMPs), leading to loss of extracellular matrix proteins such as collagen and elastin.

Aim: To understand the changes in smooth muscle specific genes and proteins in aneurysms of the ascending thoracic aorta, the involvement and relative roles of different MMPs in a large series of (n=25) ascending thoracic aneurysms (ATAs).

Patients and methods: ATA samples were provided by Kartal Koşuyolu, Advanced Training and Research Hospital. Total RNA was isolated from aneurysmal tissue. Smooth muscle cells (SMC) were isolated from 15 ATA samples using the explant technique and immunohistochemistry (IHC) was performed to characterize SMC marker proteins; SMC actin, desmin and vimentin. The expression of certain genes in aneurysmal tissue and isolated cells SMC were determined by real time PCR. SMCs from healthy subjects and nondiseased aortic tissue (n=4) were used as controls.

Results: There are significant differences between the expression of SMC contractile genes and proteins such as actin, myosin heavy chain and calponin in tissue and cells of ATA patients. Measured MMP-2,-9 and -13 gene expressions, normalized to controls also differed significantly.

Conclusion: Our results indicate that SMC in ATA tissues present with a phenotypic diversity, which persists in SMC isolated from tissue.

Supported by the EU 7th Framework Program Project “Fighting Aneurysmal Diseases” (FAD, 200647).

Poster Session

ROLE OF THE COP9 SIGNALOSOME IN NF-KAPPAB SIGNALING IN ATHEROSCLEROSIS

Yaw Asare[1,2], H. Noels[2], H. Lue[1], A. Zernecke[3], C. Weber[2], M. deWinther[4], J. Bernhagen[1]

[1]Department of Biochemistry and Molecular Cell Biology, [2]Institute for Molecular Cardiovascular Research, RWTH Aachen University, Aachen, [3]Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany, [4]Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, Netherlands Antilles

Inflammatory processes play a crucial role in all stages of atherogenesis: from early endothelial activation by modified lipids to the eventual rupture of the atherosclerotic plaque. Activation of the canonical NF-kappaB signal transduction pathway, mediated by phosphorylation and degradation of IkappaBalpha by the IkappaB-Kinase (IKK) complex, is a crucial step in pro-inflammatory target gene expression such as that of chemokines or adhesion molecules, which have been implicated in the initiation and development of atherosclerosis. The role of the COP9 signalosome (CSN), a multi-functional protein complex with functions in NF-kappaB and beta2-integrin signaling, in atherosclerosis has been unknown. Here we silenced CSN5/JAB1, a subunit of the CSN responsible for the cullin-1 deNEDDylation activity of CSN, in endothelial cells (HUVECs) using siRNA technology. IkappaBalpha levels were decreased in *CSN5* and *CSN2* knockdown cells and further experiments revealed that this decrease was due to increased cullin-1 and cullin-1-NEDD8 levels, indicating that the CSN regulates the stability of cullin-1 which in turn affects the turnover of IkappaBalpha. Next, we observed that TNFalpha-dependent degradation of IkBalpha was enhanced (52%) by *CSN5*- or *CSN2*-siRNA treatment (knockdown efficiency: ~40%) and an increase of NF-kB activity (80%). Furthermore, siRNA knockdown of *CSN5* or *CSN2* under pro-atherogenic conditions led to a prolongation of TNFalpha-induced phosphorylation of IKK. Finally, preliminary data indicate that CSN3 interacts with IKK in a TNFalpha-dependent manner. We propose that the CSN controls the activity of NF-kappaB and might play a vital role in the canonical NF-kappaB signal transduction pathway in inflammatory and atherogenic conditions.

Poster Session

INFLAMMATORY MARKERS IN PATIENTS UNDERGOING IMPLANTABLE CARDIOVERTER DEFIBRILLATOR IMPLANTATION FOR LIFE THREATENING VENTRICULAR ARRHYTHMIAS

Thomas Papadopoulos[1], N. Katsiki[2], G. Dadous[1], I. Kanonidis[1], G. Sakadamis[1]

[1]2nd Department of Cardiology, Hippokration University Hospital, Aristotle University of Thessaloniki, [2]Medical School, Aristotle University of Thessaloniki, Thessaloníki, Greece

Aim: To investigate the role of inflammation in patients with life threatening ventricular arrhythmias.

Methods: Blood samples were taken from twenty-one male patients (mean age=64±9,27yrs) undergoing ICD implantation for life-threatening ventricular arrhythmias. Samples were drawn simultaneously from three different sites i.e. the aorta, the coronary sinus and the subclavian vein and analyzed for IL-1beta, IL-6, IFN-gamma, TNF-alpha and hsCRP.The study population was divided and analyzed in two ways, according to:

1) the presence or absence of CAD, documented by coronary angiography and

2) the level of EF (< or>40%), documented by left ventriculography or MUGA.

Results: In 71,4% of the patients, levels of IL-1beta, IL-6, IFN-gamma, TNF-alpha and hsCRP were within normal values. There were not significant differences among the levels of all markers in relation to the site of blood sampling. Patients with CAD (47,6%) had higher levels of IL-1beta and IFN-gamma in the aorta and higher levels of hsCRP in all sites compared to patients without CAD.Levels of IL-1beta, IL-6, IFN-gamma, TNF-alpha and hsCRP were also higher in all sites in patients with EF< 40% (63,2%) compared to those with EF>40%. None of the observed differences was significant.

Conclusion: This is the first study to report the levels of certain inflammatory markers in three different sites in patients undergoing ICD implantation for ventricular arrhythmias. Our results do not support a clear relationship between inflammation and life-threatening ventricular arrhythmias. However, in patients with CAD or low EF a trend towards higher levels of the markers was observed, suggesting an enhanced inflammatory activity in those patients.

Poster Session

ATHEROGENIC NON-UNIFORM SHEAR STRESS INDUCES ENDOTHELIAL DYSFUNCTION AND INCREASED LEUKOCYTE RECRUITMENT IN RESPONSE TO TNF-A

Katharina Urschel[1], A. Wörner[1], K. Beronov[2], W.G. Daniel[1], C.D. Garlichs[1], I. Cicha[1]

[1]Department of Cardiology and Angiology, University of Erlangen-Nuremberg, Erlangen, [2]Bavarian Academy of Sciences and Humanities, Leibniz Supercomputing Centre, Munich, Germany

Objective: Atherosclerosis-prone regions are characterized by non-uniform shear stress patterns inducing endothelial activation. This process contributes to inflammatory cell recruitment in the arterial wall. We investigated the influence of circulating TNF-α on leukocyte recruitment and adhesion molecule expression in endothelial cells exposed to laminar versus non-uniform shear stress.

Methods: HUVECs seeded in bifurcating flow-through cell culture slides were exposed to laminar or non-uniform shear stress either with or without an additional TNF-α stimulation. Subsequently, HUVECs were perfused with medium containing THP-1 monocytes or freshly isolated PBMCs. Adherent leukocytes were quantified by light microscopy. Adhesion molecule expression was determined by immunofluorescence.

Results: Adhesion of leukocytes to unstimulated HUVECs was nearly undetectable under laminar shear stress and was slightly increased under non-uniform shear stress. Upon TNF-α stimulation, upregulation of E-selectin and VCAM-1 expression significantly increased over time (1-4 h) under non-uniform shear stress. Moreover, exposure of HUVECs to non-uniform shear stress followed by 2h stimulation with circulating TNF-α induced a 12-fold increase in THP-1 monocyte recruitment. These effects were prevented in HUVEC exposed to laminar shear stress. Essentially the same adhesion pattern was observed, when freshly isolated PMBCs were used.

The significant differences in TNF-α-induced THP-1 monocyte recruitment and adhesion molecule expression between laminar and non-uniform shear stress regions were abolished in the absence of shear stress preconditioning or by HUVEC pre-treatment with a NF-κB-inhibitor.

Conclusions: Non-uniform shear stress increases endothelial susceptibility to circulating TNF-α and induces leukocyte recruitment. Interference with this process may inhibit inflammatory response in atherosclerosis-prone regions.

Poster Session

CREATION OF A RAT EXPERIMENTAL MODEL OF ABDOMINAL AORTIC ANEURYSMS

Karina M. Mata[1], F.S. Rocha[1], P.S. Prudente[1], C.M. Prado[1], E.M. Floriano[1], J. Elias Jr[2], E. Rizzi[3], R.F. Gerlach[4], M.A. Rossi[1], S.G. Ramos[1]

[1]Pathology, [2]Radiology, [3]Pharmacology, Faculty of Medicine of Ribeirão Preto/University of São Paulo, [4]Department of Morphology, Estomatology and Physiology, Dental School of Ribeirão Preto/University of São Paulo, Ribeirão Preto, Brazil

Introduction: Animal models of abdominal aortic aneurysm (AAA) are important tools for investigating the underlying pathophysiology.

Objectives: To create of a new experimental model of AAA caused by outside traumatic vascular injury and turbulent flow in the abdominal aorta.

Method: Male Wistar rats (150g) were randomly divided into four groups: Aneurysm (A), Injury (I), Stenosis (S) and Control (C) (40/group). The (I) group received an outside traumatic injury in the aortic wall. The (S) group received an extrinsic stenosis at a corresponding location. The (A) group received both the injury and stenosis simultaneously, and the (C) group received a sham operation. Aneurysms developed in 60-70% of the animals in group (A). Animals were euthanized at days 1, 3, 7 and 15 to accompany the induction of aneurysm.

Results/conclusion: Animals developed AAA by day 3 and reached a media 8 times larger than normal. The aneurysmal wall exhibited increased thickness due to damaged and fragmented elastic fibers, mesenchymal cell proliferation, neoangiogenesis and an intense inflammatory infiltration seen within a dense collagenous matrix. We hypothesized that damaged to the vessel wall initiates an inflammatory response that further exacerbates vessel damage via the elaboration of metalloproteinases. Aortic stenosis causes turbulent flow, altering endothelial cells homeostasis and contributing to the development of aneurysms. Outside traumatic injury and disruption of endothelial cell homeostasis together cause AAA in rats. Our model illustrates that AAA is a dynamic remodeling process and will serve as an important tool for future studies of the pathophysiology of AAA.

Poster Session

FENOFIBRATE ATTENUATES NICOTINE-INDUCED VASCULAR ENDOTHELIAL DYSFUNCTION IN THE RAT

Vishal Chakkarwar, M. Singh

Cardiovascular Pharmacology Division, I.S.F. Institute of Pharmaceutical Sciences and Drug Research, Moga, India

The study has been designed to investigate the effect of fenofibrate, in nicotine-induced vascular endothelial dysfunction (VED) in rats. Nicotine (2 mg/kg/day, i.p., 4 weeks) was administered to produce VED in rats. The development of VED was assessed by employing isolated aortic ring preparation and estimating serum and aortic concentration of nitrite/nitrate. Further, the integrity of vascular endothelium was assessed using the scanning electron microscopy of thoracic aorta. Moreover, the oxidative stress was assessed by estimating serum thiobarbituric acid reactive substances (TBARS) and aortic superoxide anion generation. Furthermore, the lipid profile was assed by estimating serum cholesterol, triglycerides and high density lipoprotein. The administration of nicotine produced VED by impairing the integrity of vascular endothelium and subsequently decreasing serum and aortic nitrite/nitrate and attenuating acetylcholine-induced endothelium dependent relaxation. Further, nicotine produced oxidative stress, assessed in terms of increase in serum TBARS and aortic superoxide generation. Moreover, nicotine altered the lipid profile by increasing the serum cholesterol, triglycerides and decreasing the high density lipoprotein. However, treatment with fenofibrate (32 mg/kg, p.o.) or atorvastatin (30 mg/kg/day p.o., a standard agent) prevented nicotine-induced VED, oxidative stress and normalizes the altered lipid profile by improving integrity of vascular endothelium, increasing the serum and aortic nitrite/nitrate, enhancing the acetylcholine-induced endothelium dependent relaxation and decreasing serum TBARS and aortic superoxide anion generation. Thus, it may be concluded that fenofibrate reduces the oxidative stress, normalizes the altered lipid profile and consequently improves integrity of endothelium and enhances the generation of nitric oxide to prevent nicotine-induced experimental VED.

Poster Session

PEROXYNITRITE ACTIVATES THE TRANSCRIPTION FACTOR NRF2 IN HUMAN ENDOTHELIAL CELLS.

Laurine Mattart, D. Simon, D. Calay, V. Tevel, M. Van Steenbrugge, M. Raes

FUNDP - University of Namur, Namur, Belgium

Endothelial dysfunction is an early phenomenon in cardiovascular diseases, characterized by a decrease in bioavailable nitric oxide ($NO^{\cdot}$). This decrease further enhanced when there is a concomitant overproduction of superoxide ions ($O_2^{\cdot -}$) and $NO^{\cdot}$. It is observed during local inflammatory reaction where $NO^{\cdot}$ reacts with $O_2^{\cdot -}$ to form peroxynitrite ($ONOO^-$). Recent data suggest that $ONOO^-$ activates the Nrf2 pathway. The aim of our work was to study the response of human EAhy926 endothelial cells to $ONOO^-$ and to highlight roles of the Nrf2 pathway. For this purpose, cells were exposed to SIN-1 (3-morpholinosydnonimine) an $ONOO^-$ donor. Nrf2 was activated after SIN-1 incubation and two of its well known target genes, Heme Oxygenase 1 (*HO-1*) and NAD(P)H:quinone Oxidoreductase-1 (*NQO1*), were overexpressed at the mRNA level. On the other hand, we demonstrated that SIN-1 treatment is associated with a significant increase in LC3-II formation, resulting in autophagosome formation, and a decreased in DNA fragmentation. Interestingly, the invalidation of Nrf2 or HO-1 by siRNA strongly increased SIN-1 induced LC3-II formation and DNA fragmentation. This study show that peroxynitrite have a protective effect on endothelial cells by inducing protective pathways such as Nrf2 and autophagy.

L. Mattart and D. Calay are recipient of a FRIA fellowship - This research is supported by the IUAP program P06/30.

Poster Session

EARLY EFFECTS OF ATORVASTATIN AND CLOPIDOGREL ALONE OR COMBINED ON ENDOTHELIAL PROGENITOR CELLS AND MICROPARTICLES IN SUBJECTS WITH CORONARY DISEASE

Carolina França[1], L. Pinheiro[1], M. Izar[1], M. Brunialti[2], R. Salomão[2], F. Fonseca[1]

[1]Cardiology, [2]Infectology, Federal University of São Paulo, São Paulo, Brazil

Introduction: Atherosclerosis is an inflammatory disease complicated with thrombotic events related to endothelial dysfunction or apoptosis. Recently, the turnover of endothelial cells has been better understood with a crucial role of endothelial progenitor cells (EPC). In addition, increased amount of microparticles derived from endothelial apoptotic cells (EMP) has been reported in patients with uncontrolled risk factors. We aimed to examine early effects of statin and clopidogrel therapy in subjects with stable coronary heart disease on EPC, EMP and platelet derived microparticles (PMP) and possible pharmacokinetic interaction between these widespread used therapies.

Methods: Patients of both sexes, with mean (SD) age of 62.2 (9.11) years were included if they also had LDL-C < 100 mg/dL, under a stable dose of statin, that was stopped for 1-wk to obtain baseline levels of EPC, EMP, and PMP, quantified by flow cytometry. At this time point, atorvastatin 80 mg/d was initiated. After 1-wk we repeated the same tests and started clopidogrel 75 mg/d. After new measurements 2-wk after the combined treatment, atorvastatin was discontinuated to evaluate the effects of clopidogrel alone 1-wk later.

Results: The mean (SEM) EMP CD51+ plasma levels (microparticles/µL) were: 748 (263) at baseline; 1251 (321) 1-wk after atorvastatin; 2107 (637) 2-wk after atorvastatin+clopidogrel; and 703 (330) with clopidogrel alone. There were differences between the first and third (p=0,024) and among the third and fourth time points (p=0,007). No changes were seen for EPC or PMP.

Conclusion: The increased number of EMP with the combined treatment suggests an interaction between drugs.

Poster Session

PROFILING OF BIGLYCAN MOLECULAR PROMOTER HAPLOTYPES

Boris Schmitz[1], R. Telgmann[1], E. Brand[2], C. Rüßmann[1], J. Fischer[3], M. Paul[4], S.-M. Brand-Herrmann[1]

[1]Leibniz-Institute for Arteriosclerosis Research, University of Münster, [2]Department of Internal Medicine D, Nephrology and Hypertension, University Hospital Münster, Münster, [3]Institute of Pharmacology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [4]Faculty of Health, Medicine, and Life Science, Maastricht University, Maastricht, The Netherlands

Introduction: The small leucine rich proteoglycan biglycan (BGN) is involved in cardiovascular disease (CVD) pathophysiology. The aim of the current study was to identify and functionally characterize the differential usage of BGN gene promoter haplotypes in different cell lines.

Material and methods: We directly resequenced a 1199 bp portion of the BGN gene promoter in 57 patients with cardiovascular disease to characterize its variant structure. Molecular haplotypes (MolHaps) were determined by subcloning of individuals' DNA. MolHaps and promoter deletion constructs were subcloned and transfected into HEK293T, EA.hy926 and THP-1 monocytes. Cells were kept under basal conditions or stimulated with 10^{-8}M PMA or 0.5 mM 8-Br-cAMP for 24 hrs. Transcriptional start sites (TSS) were determined by semiquantitative PCR.

Results: We identified three SNPs in the BGN 5'-regulatory region (G-849A, G-578A, G-151A), and one in the 5'-UTR (G+94T) composing three respective MolHaps: 1 [G^{-849}-G^{-578}-G^{-151}-G^{+94}; wildtype (wt)], 2 [G^{-849}-G^{-578}-A^{-151}-T^{+94}] and 3 [G^{-849}-A^{-578}-G^{-151}-G^{+94}]. Under both basal and stimulatory conditions MolHap 2 was significantly less active than wt in HEK293T and EA.hy926. Whereas all cell lines displayed transcripts from the main TSS (position +1), EA.hy926 cells showed strong usage of an alternative TSS positioned ~45 bp upstream of the main TSS. Chromatin Immunoprecipitation assays revealed transcription factor SP1 to be involved in promoter regulation in EA.hy926 cells.

Conclusion: We were able to show that (1) BGN MolHaps exert cell type-specific differentially functional properties, (2) promoter usage obviously differs across cell lines, and (3) the 5'-UTR represents an active part of the BGN gene promoter.

Poster Session

RESPONSE OF ENDOTHELIAL CELLS TO SIMVASTATIN UNDER STATIC CULTURE, STEADY FLOW, NON-REVERSING PULSATILE FLOW AND OSCILLATING FLOW

Joanna Rossi[1,2], L. Rouleau[1,2], J.-C. Tardif[2], R.L. Leask[1,2]

[1]McGill University, [2]Montreal Heart Institute, Montreal, QC, Canada

Introduction: In addition to lowering cholesterol levels, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, have been shown to modulate gene expression in endothelial cells. It has been reported that statins can elevate the expression of Kruppel-like factor 2 (KLF2), endothelial nitric oxide synthase (eNOS), and thrombomodulin in cultured endothelial cells. However, few studies have evaluated whether wall shear stress induced by blood flow can impair or enhance endothelial cell response to statins.

Methods: Human abdominal aortic endothelial cells (HAAEC) were treated with control vehicle, simvastatin (0.1, 1 or 10 μM) or 10 μM simvastatin with 200 μM mevalonate under static culture, steady flow (18 dynes/cm^2), non-reversing pulsatile flow (18 ± 9 dynes/cm^2) and oscillating flow (0.3 ± 3 dynes/cm^2) for 24 h.

Results: Steady flow and non-reversing pulsatile flow enhanced KLF2, eNOS, and thrombomodulin mRNA, whereas oscillating flow reduced their expression. Under static culture and all three flow conditions (steady, pulsatile, and oscillating flow), simvastatin elevated KLF2, eNOS and thrombomodulin expression. The highest level of all three genes was achieved with a combination of steady laminar flow and 10 μM simvastatin. The effect of simvastatin was completely eliminated when the cells were treated with 10 μM simvastatin and 200 μM mevalonate.

Conclusions: Simvastatin enhances KLF2, eNOS, and thrombomodulin expression in cultured endothelial cells regardless of the external fluid dynamic environment through inhibition of mevalonate synthesis. These findings may explain the observed non-lipid lowering benefits of statins in the clinic.

Poster Session

ENDOGLIN (TYPE III TGF-B1 RECEPTOR), BAD OR GOOD IN ATHEROSCLEROSIS?

Petr Nachtigal[1], Z. Strasky[1], L. Vecerova[1], E. Brcakova[1], M. Slanarova[1], J. Rathouska[1], S. Micuda[2]

[1]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Biological and Medical Sciences,
[2]Charles University in Prague, Faculty of Medicine, Department of Pharmacology, Hradec Kralove, Czech Republic

Endoglin is a homodimeric transmembrane glycoprotein that is a part of transforming growth factor-β (TGF-β) receptor cascade. It has been demonstrated that endoglin can functionally interact with TGF-βRI (ALK-1) to activate Smad1/5 or with the TGF-βRI (ALK-5) to activate Smad2/3/eNOS expression. In this study we hypothesized whether atorvastatin can affect endoglin/SMAD1,5,8/SMAD2/eNOS signaling in the mice aortic atherosclerotic plaques.

Two-month-old female apoE/LDLr-deficient mice were divided into two groups. The control group (n=8) was fed with the cholesterol rich diet (0.15% of cholesterol) whereas atorvastatin at dose 50 mg/kg per day was added to the same diet in atorvastatin group (n=8). Biochemical analysis of blood, plaque size area and western blot analysis of endoglin, p-SMAD1,5,8, p-SMAD2 and p-eNOS expressions in aorta were performed.

The biochemical analysis showed that administration of atorvastatin significantly decreased level of total cholesterol, VLDL, LDL and atherosclerotic lesion area when compared with control mice. On the contrary western blot analysis revealed induced endoglin, p-SMAD2, p-SMAD1,5,8 and p-eNOS expression in atorvastatin treated mice when compared with control mice.

In conclusion, in this study we showed that hypolipidemic and antiatherogenic effects of atorvastatin are related to significant increase of endoglin/SMAD1,5,8/SMAD2/eNOS pathway suggesting potential antiatherogenic effect of endoglin and SMADs in atherosclerosis.

This work was supported by grant from The Grant Agency of Charles University in Prague number 129208/C, grant MSM 002162082 and Research project MZO 001799060.

Poster Session

EXPRESSION OF MATRIX METALLOPROTEINASES AND MECHANISM OF EXTRACELLULAR MATRIX REMODELING IN TAKAYASU'S ARTERITIS

Nitin Mahajan[1], S. Malik[1], S. Jain[2], V. Dhawan[1]

[1]Exp Med & Biotech, [2]Internal Medicine, PGIMER, Chandigarh, India

Introduction: Takayasu's arteritis is an inflammatory fibrosing arteritis affecting predominately the aorta and its main branches. Pathogenesis of this disease remains enigmatic. Despite the numerous studies the role of adventitia in vascular lesion formation in the setting of TA has been ignored. Virtually nothing is known about the mechanism regulating inflammation in the adventitia in the setting of TA.

Methods: Present study included subjects with Takayasu's arteritis (Gr.I) and normal healthy control subjects (Gr. II). T-cells were isolated from the PBMCs using nylon wool and stimulated with PMA for 24 Hrs. Stimulated cell were fixed and fractionated into membrane, cytosolic and nuclear fractions. These cellular fractions were co-cultured with human fibrosarcoma cell line (HT1080) and transcriptional expression of matrix metalloproteinases (MMPs) was determined using semi-quantitative RT-PCR.

Results: Upon contact with fixed activated T-lymphocytes, a massive induction of the expression of MMPs and their tissue inhibitor (TIMP-1) was observed, whereas, unstimulated T-cells and their subfractions had no effect. Stimulation of MMPs-TIMP synthesis by HT1080 cells was mimicked by a membranous preparation derived from activated T-cell, whereas, cytosolic and nuclear fractions were ineffective.

Conclusions: Cell-cell contact may represent an important biological mechanism for potentiating the inflammatory response that leads to extracellular matrix degradation. Present study provides a directive evidence for the presence of a cell surface specific antigenic moiety on T-cells of TA subjects. This antigenic stimulation may be responsible for activation of fibroblast to enhance MMP production and requires characterization in near future.

Poster Session

BCL-XL INACTIVATION IN MACROPHAGES IS ASSOCIATED WITH ACCELERATED PROGRESSION IN ADVANCED ATHEROSCLEROTIC LESIONS OF APOE-/- MICE

Andrew Shearn, V. Deswaerte, J. Pirault, F. Saint Charles, M.J. Chapman, E.L. Gautier, T. Huby, P. Lesnik

INSERM UMR S939, Paris, France

Atherosclerosis is an inflammatory disease that is associated with monocyte recruitment, subsequent differentiation into lipid-laden macrophages and ultimately macrophage death. Bcl-xL is the most abundantly expressed member of the Bcl-2 gene family in macrophages and plays a protective role against macrophage apoptosis. However, the role of Bcl-xL in atherosclerosis is currently unknown. We therefore specifically inactivated Bcl-xL in macrophages and determined the impact on atherosclerotic lesion formation in an $apoe^{KO}$ background. $Bcl\text{-}xL^{KOmac}$ ($Bcl\text{-}xL^{flox}$-$LysM^{cre}$, $apoe^{KO}$) mice fed a Western diet for 27 weeks exhibited advanced lesions in the whole aorta which were 45% larger than those seen in control mice; these changes were associated with elevated liver and plasma cholesterol levels. Analysis of liver tissue by flow cytometry revealed a significant decrease (33%, $p< 0.001$) in resident macrophages (Küpffer cells) in $Bcl\text{-}xL^{KOmac}$ mice in comparison to controls. Our data suggest that modulation of macrophage resistance to apoptosis through targeted deletion of Bcl-xL may impact the macrophage cell population in the whole body, including Küpffer cells, the largest pool. Macrophage survival may not only influence atherosclerotic plaque development, but also, lipid and hepatic metabolism.

Poster Session

ROLE OF DIFFERENT FORMS OF HDL (OX-HDL AND N HDL) ON OX-LDL INDUCED APOPTOSIS IN DIFFERENTIATED MONOCYTES

Navkiran Kaur[1], S. Kumari[1], S. Jain[1], S. Majumdar[2], S. Ghosh[2]

[1]Department of Internal Medicine, [2]Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh, India

Introduction: Macrophages in atherosclerotic lesions have been shown to have cellular characteristic of both necrosis and apoptosis. However, apoptosis of macrophages is beneficial or detrimental during atherosclerosis is not well understood.

Objectives: This study examined the role of different forms of HDL (nHDL/Ox-HDL) on mitochondrial dependent apoptotic pathway induced by Ox-LDL in differentiated monocytes.

Methods: Apoptosis was examined in PMA differentiated monocytes obtained from THP-1 cells as well as 30 hyperlowdensitylipoproteinemic and normolowdensitylipoproteinemic subjects which were preincubated with nHDL/Ox-HDL followed by coincubation with Ox-LDL and further the expression of various pro- and anti- apoptotic molecules was studied both mRNA and protein level.

Results: We demonstrated that nHDL but not Ox-HDL inhibit Ox-LDL mediated apoptosis in differentiated monocytes. The findings were substantiated in patient and control subject by studying the expression of Bax and Bcl-2 proteins.In the present study,Ox-LDL and Ox-HDL were found to be cytotoxic in differentiated monocytes in a dose dependent manner. HDL is also susceptible to oxidation and this modification of HDL leads to the cytotoxicity in cultured cells. The Ox-HDL also induced apoptosis of differentiated monocytes/macrophages in presence of Ox-LDL via activation of Bax leading to mitochondrial dysfunctioing and release of cytochrome C in the cytosol.

Conclusion: HDL is identified as carrier of monocytes/ macrophage survival factor and suggests that induction of mitochondrial pathway of apoptosis of monocytes/macrophage by Ox-HDL may represent an important and novel aspect of HDL indicating that modification of lipoprotein like HDL may enhance the process of atherogenesis.

Poster Session

SEX PARTICULARITIES IN NITRIC OXIDE AVAILABILITY IN HEALTHY AND HYPERTENSIVE RATS UNDER NORMAL AND STRESS CONDITIONS

Antonina Kuznetsova, O. Semychkina-Glushkovskaya, T. Sindyakova, I. Semyachkin-Glushkovskij, Y. Kuznetsova

Biology Department, Saratov State University, Saratov, Russia

Aims: The goal of this study was to determine the gender differences in nitric oxide (NO) content in normal and hypertensive (two kidney, one clip) rats at rest and in immobilization stress (IS, 60 min).

Methods: Serum NO concentration were measured in blood by a spectrophotometric assay. NO determinations were performed by Gress reaction as NO_2 concentration after NO_3 reduction to NO_2.

Results: We found that the basal NO production was greater in females vs. males. The IS resulted in significant increase in circulating NO levels that were more pronounced in females vs. males. The ischemia of renal artery was accompanied by development of hypertension and atrophical decrease in mass of kidney. Actually, the hypertension was attenuated in females compared with males. So, the basal BP levels were higher in hypertensive males vs. hypertensive females. The mass of clipped kidney vs. mass of normal kidney was decreased 8.3-fold in females and 15.8-fold in males. We also found that NO production was depressed in hypertensive rats under normal and stress conditions. Nitice, that severe hypertension in males vs. females was accompanied by more significant decrease in basal and stress NO secretion.

Conclusion: The present study shows that there are sex differences in NO availability both normotensive and hypertensive rats under normal and stress conditions. Since NO is a vasorelaxing and important stress-limited factor we hypothesize that there are sex differences in basal and stress NO availability may be responsible for man are at greater risk for cardiovascular disease than women. Project GK-P1257.

Poster Session

A NOVEL APPROACH THROUGH THE EFFECTS OF TURBULENT FLOW ON THE ENDOTHELIAL CELL SHAPE

Reza Paeizi, E. Barzanouni, M. Bokaiyan

Mashhad University of Medical Sciences, Mashhad, Iran

Turbulent flow is an aggregator of injury and dysfunction of endothelial cells lining which leads to deposition of low density lipoprotein (LDL) into the intima layer of large and medium sized elastic and muscular arteries; chronic deposition of LDL leads to progression of atherosclerosis plaque formation. Clinical evidences show atherosclerosis occurs in bifurcation and curvatures which are concerning regions of our hypothesis due to different flow pattern called turbulent flow; through a new point of view, we approach the possible role of turbulent blood flow and endothelial luminal cell surfaces which induces static electricity on plasma membrane; this static electricity can cause a new arrangement of actin filaments- cell shape determiners - which can induce chronic shifting of cytoskeleton and chronic changing in cell shape. Changing process effects cell-to-cell junction and leads to concordance disruption in lining of endothelial cells. This phenomenon can cause endothelial dysfunction of arterial lining which might cause chronic events that leads to atherosclerosis. Taken together, turbulent flow results in atherosclerosis with induction of static electricity which can help us design more efficient prophylactic and therapeutic strategies.

Poster Session

THE BENEFICIAL EFFECT OF MEDITERRANEAN DIET ON METABOLIC SYNDROME: A META-ANALYSIS OF 49 STUDIES AND 175,529 INDIVIDUALS

Christina-Maria Kastorini[1], H. Milionis[2], K. Esposito[3], D. Giugliano[3], J. Goudevenos[2], D.B. Panagiotakos[1]

[1]Department of Nutrition-Dietetics, Harokopio University, Athens, [2]School of Medicine, University of Ioannina, Ioannina, Greece, [3]Department of Geriatrics and Metabolic Diseases, Second University of Naples, Naples, Italy

Objective: To systematically review and meta-analyze epidemiological studies and clinical trials that have assessed the effect of a Mediterranean diet on Metabolic syndrome and its components.

Design: Systematic review and random effects meta-analysis of epidemiological studies and randomized controlled trials. English language publications in PubMed, Embase, Web of Science and the Cochrane Central Register of Controlled Trials from 1/1/1990 to 31/12/2009; 49 original research studies (35 clinical trials, 2 prospective and 12 cross-sectional), with 175,529 participants, were included in the analysis.

Results: The overall effect shows that adherence to the Mediterranean diet is associated with reduced odds of metabolic syndrome (log (OR): -0.36, 95%CI: -0.63 to -0.09). Additionally results from clinical studies (mean difference, 95%CI) indicate the protective role of the Mediterranean diet on metabolic syndrome components: waist circumference: -0.42 cm, 95%CI: -0.82 to -0.02, HDL: 1.17 mg/dL, 95%CI: 0.38 to 1.96, triglycerides: -6.14 mg/dL, 95%CI: -10.35 to -1.93, systolic (SBP): -2.35 mmHg, 95%CI: -3.51 to -1.18 and diastolic blood pressure (DBP): -1.58 mmHg, 95%CI: -2.02 to -1.13, glucose: -3.89 mg/dL, 95%CI:-5.84 to -1.95. Results from epidemiological studies confirm those of clinical trials: waist circumference: -4.87 cm, 95%CI: -6.21 to -3.53, HDL: 2.04 mg/dL, 95%CI: 1.01 to 3.07, triglycerides: -8.63 mg/dL, 95%CI: -14.89 to -2.36, SBP: -2.19 mmHg, 95%CI: -4.91 to 0.54, DBP: -2.34 mmHg, 95%CI: -5.54 to 0.86, glucose: -3.32 mg/dL, 95%CI: -5.90 to -0.74.

Conclusions: The present meta-analysis suggests that Mediterranean diet is beneficial not only regarding metabolic syndrome, as a whole, but also its individual components.

Poster Session

MIPOMERSEN, AN APO B SYNTHESIS INHIBITOR, PREFERENTIALLY REDUCES SMALL LDL PARTICLE NUMBER AND INCREASES LDL PARTICLE SIZE IN HOFH PATIENTS

William Cromwell[1], R. Santos[2], D. Blom[3], A.D. Marais[3], M.-J. Charng[4], R. Lachmann[5], D. Gaudet[6], J.-L. Tan[7], D. Tribble[8], J. Flaim[8], S. Chasan-Taber[9], J. Donovan[9], F. Raal[10]

[1]Division of Atherosclerosis and Lipoprotein Disorders, Presbyterian Cardiovascular Institute, Charlotte, NC, USA, [2]Heart Institute (inCor), University of São Paulo, São Paulo, Brazil, [3]Division of Lipidology, Department of Medicine, University of Cape Town, Cape Town, South Africa, [4]Department of Cardiology, Taipei Veterans Genl Hosp and Natl Yang-Ming Univ, Taipei, Taiwan R.O.C., [5]Charles Dent Metabolic Unit, University College London Hospitals, London, UK, [6]ECOGENE-21 Clinical Research Ctr and Lipid Clinic, Chicoutimi, QC, Canada, [7]Cardiology, National Heart Center, Singapore, Singapore, [8]Isis Pharmaceuticals, Inc., Carlsbad, CA, [9]Genzyme Corporation, Cambridge, MA, USA, [10]Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa

Background and Aim: Apolipoprotein B (apo B) is a key component of atherogenic lipoprotein particles VLDL, IDL, and LDL, elevations of which are associated with increased cardiovascular risk. Mipomersen (MIPO, ISIS 301012), an investigational apo B synthesis inhibitor, was evaluated in a Phase 3 study involving 51 patients with homozygous familial hypercholesterolemia (HoFH) randomized to 26 weeks placebo (PBO, n=17) or MIPO 200 mg/wk (n=34) s.c. added to ongoing lipid-lowering therapy. MIPO produced a 25% mean reduction in LDL-C and reductions in apo B, TC, and non-HDL-C (all p< 0.001) (Raal, Lancet, 2010). Herein, we report lipoprotein particle numbers (lipoprotein-P) and subclass distributions based on NMR in this largest known HoFH NMR dataset.

Results: Baseline [total, nmol/L, mean (SD)] VLDL-P [PBO: 102.68 (46.49); MIPO 133.95 (91.04] and LDL-P [PBO: 2689.7 (1226.6); MIPO 3088.5 (1151.7)] were high and LDL size [nm, mean (SD), PBO: 21.91 (0.68); MIPO 21.72 (0.89)] was large relative to other populations. MIPO reduced VLDL-P [mean (SD): PBO: 7.18(43.75) vs. MIPO -48.47(73.91), p=0.016], mainly due to reduction in small VLDL fractions: [PBO: 0.34(28.14); MIPO -32.14(45.12), p=0.030] and LDL-P [PBO: 196.8(629.0); MIPO -1004.2(823.5), p< 0.001] mainly due to reduction in small LDL fractions [PBO: 223.1(823.6); MIPO -741.8(881.8), p< 0.001] with modest reductions in large LDL-P and IDL-P. VLDL size did not change whereas LDL size increased [PBO: -0.13(0.52); MIPO 0.47(0.60), p=0.002].

Conclusion: Given the association of increased LDL particle number with CV risk (Cromwell, *Clin Lipidology.* 2007) mipomersen-induced reductions in total and small LDL particle number warrant further investigation.

Poster Session

ANTIOXIDATIVE ACTIVITY OF SMALL DENSE HDL3 IS ENHANCED IN HETEROZYGOUS CETP DEFICIENCY : A CASE REPORT FROM A DUTCH FAMILY

Sandrine Chantepie[1], A. Klerkx[2], J.A. Kuivenhoven[2], G.K. Hovingh[2], M.J. Chapman[1], A. Kontush[1]

[1]UMRS 939, Paris, France, [2]Academic Medical Center, Amsterdam, The Netherlands

CETP deficiency results in elevated HDL-cholesterol (HDL-C) levels; its impact on HDL functionality however remains controversial.

Functional and compositional properties of HDL of 3 Dutch females with heterozygous CETP deficiency (splice-site mutation resulting in premature truncation) and HDL-C levels >90th percentile in the normal HDL-C distribution were compared with those of 3 age-matched female normolipidemic controls with mean HDL-C levels approximating the 50th percentile from the same family. As expected, HDL-C levels were increased 1.8-fold, and CETP mass and activity were decreased by -33% ($p< 0.06$) and -44% ($p< 0.05$) respectively, in carriers versus non-carriers. HDL particles from carriers were enriched in CE (up to +30%, $p< 0.01$) and depleted of triglycerides (TG; up to -75%, $p< 0.001$), resulting in a reduced TG/CE ratio (up to 4.7-fold, $p< 0.01$ vs. controls). In parallel, the apo A-I/A-II ratio was increased in HDL from carriers (up to +33%, $p< 0.05$, vs. controls). Small dense HDL3 from CETP-deficient subjects displayed enhanced antioxidative activity by attenuating LDL oxidation on a mass basis : HDL3c of carriers decreased the oxidation rate in the propagation phase of LDL oxidation more potently than HDL3c from controls (-41%, $p< 0.001$). Moreover, HDL3 from CETP-deficient subjects markedly prolonged the propagation phase relative to control HDL3 (up to +74%, $p< 0.001$).

Clearly, heterozygous CETP deficiency features pronounced changes in the lipidome and proteome of small, dense HDL which are translated into enhanced antioxidative activity. Our findings support the contention that HDL is functional in heterozygous CETP deficiency.

Poster Session

IS LYSOPHOSPHATYDYLCOLINE INVOLVED IN APOPTOSIS AND IN DEFECTIVE EFFEROCYTOSIS IN ADVANCED ATHEROSCLEROTIC PLAQUES?

Ulisse Garbin[1], E. Baggio[2], C. Stranieri[1], G. Lipari[2], S. Manfro[1], A. Pasini[1], C. Mozzini[1], A. Fratta Pasini[1], L. Cominacini[1]

[1]Internal Medicine, [2]Vascular Surgery, University of Verona, Verona, Italy

Objectives: Observational studies of advanced atherosclerotic lesions have shown that apoptotic macrophages accumulate in focal areas surrounding the developing necrotic core. The fact that necrotic cores contain predominantly macrophage debris has given rise to the concept that plaque necrosis develops as a direct consequence of postapoptotic macrophage necrosis that depends to a large extent on the efficiency of apoptotic cell clearance by phagocytes, a process known as efferocytosis. In this study we investigated the relationships between lysophosphatidylcholine (LysoPC), apoptosis and efferocytosis in human carotid endoarteriectomy specimens (HCS).

Methods and Results: HCS (n=52) were cut into different segments surrounding the lipid core (LC) and at the periphery (PS). In the different segments LysopC, apotosis and the tyrosine kinase receptors Axl, Tyro3, Mertk and the phosphatidylserine-binding protein growth arrest-specific gene 6 (GAS6) were analyzed by HPLC-mass spectrometry, Apo Tag immunohistochemical staining, western blotting and real-time PCR. LC segments had the highest LysoPC values and lipoprotein-associated phospholipase A2 (Lp-PLA2) expression ($P < 0.01$). Similarly, apoptosis was much higher in LC than PS ($p < 0.01$). On the contrary, Axl and GAS6 (both involved in efferocytosis) were significantly lower in LC than PS ($P < 0.01$). In in vitro studies increasing amounts of LysopC induced apoptosis and reduced Axl and GAS6 expression in THP-1 cells ($p < 0.01$).

Conclusions: In LC, the highest levels of LysoPC were associated to the highest degree of apoptosis and to a contemporary decline of some tyrosine kinase receptors involved in efferocytosis. The results suggest that LysoPC may be involved in defective efferocytosis of advanced atherosclerotic plaques.

Poster Session

NEW INSIGHTS IN FOAM CELL FORMATION. THE ANTIOXIDANT KEY REGULATORY FACTOR NRF2 DIRECTLY MODULATES FABP4 EXPRESSION

Iolanda Lazaro, A. Cabre, J. Girona, L. Masana

Unitat de Medicina Vascular i Metabolisme, Unitat de Recerca de Lípids i Arteriosclerosi, Hospital Universitari Sant Joan, IISPV, Universitat Rovira i Virgili. CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Reus, Spain

Background and Aims. Recently, it has been shown that oxLDL induces the expression of the adipocyte fatty acid-binding protein (FABP4) in human macrophages being one of the major contributors to foam cell formation. We have tested the hypothesis that aldehydes (2,4-DDE), molecules derived from LDL oxidation, induce FABP4 expression in THP-1 by the activation of the antioxidant cell machinery, mainly through the Nrf2 key pathway.Methods and results. Using reverse transcription and real time-PCR and Western blotting, we found that DDE produced a markedly increase in FABP4 expression at mRNA and protein levels. mRNA increase caused by DDE was abolished by both protein synthesis and transcription inhibitor agents. Moreover, DDE also affected FABP4 transcription by increasing its mRNA stability. We identified in silico a new putative antioxidant response element (ARE) site in the FABP4 gene promoter. AREs are the binding sites for the main antioxidant defence transcription factor, Nrf2. Chromatin immunoprecipitation assays confirmed Nrf2 binding to this ARE in macrophages in vivo. DDE consistently increased the activated phosphorylated form of Nrf2 in the nucleus by which de novo synthesis of Nrf2 was necessary. In addition, DDE also affected two different kinase pathways implicated in Nrf2 activation. We observed that phosphorylated forms of Akt, its downstream target GSK-3β and ERK-MAPK were increased.Conclusions. We propose a novel role of Nrf2-ARE pathway as a triggering step on foam cell formation mediated by FABP4 induction in response to oxidation. These results open new putative therapeutic targets addressed to control arteriosclerosis development.

Memory Stick

REGULAR EXERCISE IMPROVED ENDOTHELIAL DYSFUNCTION IN YOUNG MALE ADULTS WITH METABOLIC SYNDROME

Gabriel Fornieles-Gonzalez[1], M.A. Rosety-Rodriguez[2], I. Rosety-Rodriguez[2], F.J. Ordoñez[2], N. Garcia-Gomez[3], A. Camacho[4], M. Rosety-Rodriguez[1]

[1]Internal Medicine, [2]School of Sport Medicine, [3]Pathologic Anatomy, University of Cadiz, Cadiz, [4]Medicine, Hospital Juan Ramon Jimenez, Huelva, Spain

Background and aims: It is widely accepted endothelial dysfunction may play an important role during the development of atherosclerotic plaque. Recent studies have reported regular exercise may reduce proinflammatory biomarkers in patients with metabolic syndrome However to date little information is available on the influence of exercise on levels of endothelial adhesion molecules.

This study was designed to determine the influence of exercise on soluble vascular cell adhesion molecule (VCAM-1) in young male adults with metabolic syndrome.

Method: Sixty adult men with metabolic syndrome according to the criteria reported by the National Cholesterol Education Program Adult Treatment Panel III volunteered for this study. Forty-five were randomly included in experimental group to perform a 12-week aerobic training program, 3 days/week, consisting of warm up (10-min), main part (20-35-min [increasing 5 minutes each 3 weeks]) at a work intensity of 60-75% of peak heart rate (increasing 5% each 3 weeks) and cool-down (10-min). Control group included 15 age, sex and BMI-matched men with metabolic syndrome that will not perform any program. Further our protocol was approved by an institutional ethic committee. Serum soluble VCAM-1 concentration was measured by ELISA, using a commercially available kit (Parameter, R&D Systems) twice: 72-hours before starting the program and after its ending.

Results: When compared to baseline soluble VCAM-1 concentration was decreased significantly after our 6-week protocol (456.1±18.4 vs 382.7±20.3 ng/ml; $p < 0.05$). No changes were reported in controls.

Conclusion: A 12-week training program decreased soluble VCAM-1 concentration in young male adults with metabolic syndrome.

Memory Stick

EFFECTS OF SIBUTRAMINE PLUS L-CARNITINE COMPARED TO SIBUTRAMINE OR PLACEBO ON INFLAMMATORY PARAMETERS

Giuseppe Derosa[1], I. Ferrari[1], P. Maffioli[1], S. Salvadeo[1], A. Gravina[1], R. Mereu[1], A. D'Angelo[1], I. Palumbo[1], S. Randazzo[1], A. Cicero[2]

[1]University of Pavia, Pavia, [2]University of Bologna, Bologna, Italy

Aim: To evaluate the effects of one year treatment with sibutramine plus L-carnitine compared to sibutramine or placebo on body weight, lipid profile, insulin resistance, and on inflammatory state in type 2 diabetic patients.

Material and methods: Three hundred and seventy-five diabetics were randomised to take sibutramine 10 mg plus L-carnitine 2 g or sibutramine 10 mg alone or placebo alone. We evaluated at baseline, and after 3, 6, 9, and 12 months these parameters: body weight, homeostasis model assessment insulin resistance index (HOMA-IR), fasting plasma insulin (FPI), lipid profile, adiponectin (ADN), tumor necrosis factor-alfa (TNF-alfa), resistin, and high sensitivity-C reactive protein (Hs-CRP).

Results: After 12 months of treatment we observed a better improvement of body weight with sibutramine plus L-carnitine compared to the other treatments. Regarding insulin resistance the best improvement of HOMA-IR, and FPI was obtained with sibutramine plus L-carnitine. An improvement of total cholesterol and triglycerides was observed with sibutramine and with sibutramine plus L-carnitine even if with sibutramine plus L-carnitine there was also an improvement of LDL-C. A similar improvement of resistin, TNF-alfa, and Hs-CRP was obtained with sibutramine and with sibutramine plus L-carnitine, while a better improvement of ADN was recorded with sibutramine plus L-carnitine.

Conclusions: Sibutramine and L-carnitine gave a better improvement of lipid profile and of inflammatory parameters compared to placebo even if sibutramine plus L-carnitine had a better effect on HOMA-IR, FPI, LDL-C, ADN and body weight compared to sibutramine alone.

Memory Stick

BLOOD PRESSURE-LOWERING RESPONSE TO AMLODIPINE AS A DETERMINANT OF THE ANTIOXIDATIVE ACTIVITY OF SMALL, DENSE HDL3 IN HYPERTENSIVE SUBJECTS

Boris Hansel[1], X. Girerd[2], D. Bonnefont-Rousselot[2], R. Bittar[2], S. Chantepie[1], A. Orsoni[1], E. Bruckert[2], M.J. Chapman[1], A. Kontush[1]

[1]INSERM Unit 939, [2]AP-HP Pitie-Salpetriere, Paris, France

Objective: Beneficial effects of calcium channel blockers on cardiovascular disease may in part be related to the reduction of oxidative stress, potentially enhancing antioxidative activity (AOX) of high-density lipoprotein (HDL). We assessed the effect of a one-month treatment by amlodipine on HDL AOX in hypertensive subjects.

Methods: 28 hypertensive (obesity=46%; metabolic syndrome=57%) patients received 10 mg Amlodipine daily during 1 month. HDL AOX was assessed as the capacity of the small, dense HDL3c subpopulation to inhibit LDL oxidation.

Results: HDL3c AOX did not significantly change in the whole study population; changes in AOX were however positively correlated with changes in SBP ($r=0.37$, $p=0.05$ for maximal diene concentration; $r=0.34$, $p=0.08$ for LDL oxidation rate). When population was divided in two subgroups according to the blood pressure response to Amlodipine, good responders having SBP/DBP drop above the median (22/10mmHg) and poor responders below, HDL3c AOX was significantly improved in good responders vs. poor responders (change in the LDL oxidation rate in the presence of HDL3c, -6.8% (11.2) vs +1.9% (5.2) respectively, $p=0.04$; maximal diene concentration, -8.6% (13.0) vs +1.9% (8.2) respectively, $p< 0.05$). By contrast, changes in plasma concentration of oxidized LDL, a marker of systemic oxidative stress, and in chemical composition of HDL3c did not differ between the subgroups.

Conclusions: In hypertensive patients, treatment with Amlodipine resulted in the enhancement of HDL AOX only in subjects with the over-the-median decrease in blood pressure, an effect which appears to be secondary to the hypotensive effect of the drug.

Memory Stick

CHRONIC INFLAMMATORY STATE PRESENT IN FAMILIAL HYPERCHOLESTEROLEMIA IS REDUCED AFTER A FAT OVERLOAD RICH IN UNSATURATED FATTY ACIDS

Sergio Martinez-Hervas[1,2,3], C. Ivorra[3], T. Pedro[4], V. Gonzalez-Albert[3], R. Hilario[3], J.F. Ascaso[1,2], R. Carmena[1,2], J.T. Real[1,2], F.J. Chaves[3]

[1]Endocrinology and Nutrition, Hospital Clinico Universitario de Valencia, Valencia, [2]Ciber de Diabetes y Enfermedades Metabolicas Asociadas, CIBERDEM, Barcelona, [3]Unidad de Genotipado y Diagnostico Genetico, Fundacion para la Investigacion, Instituto INCLIVA, Hospital Clinico Universitario de Valencia, Valencia, [4]Endocrinology and Nutrition, Hospital Marina Alta, Denia, Spain

Introduction: Alterations in inflammation and postprandial lipemia have been involved in the development of atherosclerosis. Familial hypercholesterolemia (FH) is a primary dyslipidemia with high risk of atherosclerosis and coronary disease. However, few data is available about the response of markers of inflammation to a fat overload.

Objective: The aim of this study was to examine the effects of a fat load rich in mono and polyunsaturated fatty acids in inflammatory markers along postprandial state in patients with FH and in healthy subjects.

Methods: We studied 14 FH patients and 22 normocholesterolemic controls underwent a fat load (Supracal, SHS International). Blood samples were obtained before the fat overload (time 0) and at regular time intervals up to 8 hours (2, 4, 6 and 8 h) after the administration of the liquid load. Glucose, insulin, lipid values, and inflammatory markers, (IL1A, IL4, IL6, IL17, EGF, eotaxin, VEGF, mIp1-α, and MIP1-β), were measured.

Results: Although tryglicerides rise after fat overload, a significant decrease in most of the inflammatory markers (IL17, EGF, eotaxin, VEGF, mIp1-α, and MIP1-β) was detected. Moreover the decrease was higher in hypercholesterolemic patients than controls.

Conclusions: This study reports that a fat load enriched in monounsaturated fatty acids reduces the levels of low grade inflammatory markers related to atherosclerosis in controls and hypercholesterolemic patients, being the decrease higher in hypercholesterolemic patients. These results reinforce the notion that the unsaturated fatty acids can improve the inflammatory patterns in hypercholesterolemia and could be useful to reduce cardiovascular risk of these patients.

Memory Stick

***THE HAMBURG STUDY* - A PROSPECTIVE OBSERVATION OF 2000 HEALTHY WORKERS TO STUDY THE DEVELOPMENT OF THE METABOLIC SYNDROME**

Ulrike Beisiegel[1], K. Wegscheider[2], B. Löwe[3], C. Altenburg[1], B. Sawitzky-Rose[4], I. Zorn[1], L. Scheja[5], J. Gebhard[6], D. Müller-Wieland[4]

[1]Department of Biochemistry and Molecular Biology II, [2]Department of Medical Biometry and Epidemiology, [3]Department of Psychosomatic Medicine and Psychotherapy, University Medical Center Hamburg-Eppendorf, [4]Department of General Internal Medicine and Institute for Diabetes Research, Asklepios Clinic St. Georg, [5]Institut für Biochemie und Molekularbiologie II, Universitätsklinikum Hamburg-Eppendorf, [6]Medizinischer Dienst, Deutsche Lufthansa AG, Hamburg, Germany

Objective: To better understand the development of the metabolic syndrome (MS), we designed a cohort study including 2000 healthy workers who will be followed over a period of nine years. The objective is to study those individuals who start with one or two of the 5 classification criteria of MS and to learn about the potentially different pathophysiology, depending on the first appearing abnormalities.

Methods: Healthy workers are currently recruited at Lufthansa Technik aircraft services in Hamburg. At inclusion we investigate criteria of MS and many additional parameters of glucose as well as lipid metabolism. Furthermore, participants complete a nutritional (FFQ) and a psycho-social questionnaire and will be revisited after 3, 6 and 9 years.

Results: To date, a total of 1745 participants were recruited and characterized. The recruitment will be finalized in February 2010. The cohort consists of 1219 males and 526 females with a mean age of 42.9. In the cohort we found 630 individuals with exactly 1 feature of the MS, most of these present with elevated blood pressure. Two parameters were found in 361 participants and there the combination of blood pressure and waist circumference is most frequent. 303 individuals already fulfilled 3 or more criteria of MS.

Conclusion: The Hamburg Study has the potential to improve the understanding of the early processes in the development of the MS and to discover novel biomarkers for early risk detection.

Memory Stick

ROLE OF BUTYROPHILIN IN LIPID DROPLET SECRETION

Anneke Ruebel, I. Buers, H. Robenek

Leibniz-Institute for Arteriosclerosis Research, Muenster, Germany

Introduction: Foam cell formation is characterized by the accumulation of lipid droplets (LD) during atherogenesis. A mechanism to secrete LD is only known for the secretion of milk LD by mammary epithelial cells. Secretion of milk LD should be mediated by a tripartite complex between butyrophilin (BTN), xanthine oxidoreductase (XOR) and adipophilin. The aim of this study is to clarify the mechanism of milk LD secretion for antagonizing the accumulation of lipids in atherogenesis.

Material and results: Freeze-fracture cytochemistry approves that BTN is detectable on the outer bilayer of the plasma membrane and on the monolayer of the lipid droplet. Furthermore BTN is arranged in a network of ridges in the plasma membrane bilayer that are tightly apposed to the LD monolayer. However, adipophilin is primarily associated in the LD monolayer and not arranged in a network of ridges, this also applies to XOR. Due to our results, an interaction complex between BTN-XOR-adipophilin seems not to be possible.

Conclusion: We suppose that milk LD secretion is controlled by interactions between plasma membrane BTN and BTN in the LD monolayer. To verify our secretion mechanism, we will express BTN and fragments of BTN in epithelial cells to examine the interacting domains of BTN with a view to transfer this mechanism to macrophages. If the expression of BTN will be successful in macrophages, the possibility of inducing LD secretion and inhibiting accumulation of lipids in macrophage derived foam cells seems feasible.

Memory Stick

SERUM APOLIPOPROTEIN B48 LEVELS UNDER FASTING AND POSTPRANDIAL CONDITIONS IN WOMEN WITH AND WITHOUT POLYCYSTIC OVARIAN SYNDROME

Anne McGowan[1], T. Kyaw Tun[1], N. Correia[1], N. Phelan[1], H. Roche[2], G. Boran[3], J. Gibney[1]

[1]Department of Diabetes and Endocrinology, Adelaide and Meath Hospital, Incorporating the National Children's Hospital, [2]Nutrigenomics Research Group, Conway Institute of Biomolecular and Biomedical Research, [3]Chemical Pathology, Adelaide and Meath Hospital, Incorporating the National Children's Hospital, Dublin, Ireland

Women with polycystic ovarian syndrome (PCOS) have an atherogenic lipid profile with greater triglyceride and lower HDL cholesterol levels compared to normal subjects matched for age and BMI. The paucity of data concerning chylomicron metabolism in PCOS is at least partly due to lack of availability of a standardised method for quantification of apolipoprotein(apo)B48, which is specific for intestinally-derived lipoproteins.

The aim of this study was, using a novel ELISA method, to evaluate fasting and postprandial apoB48 concentrations in women with and without PCOS. Plasma triglyceride and ApoB48 levels were measured before and 2, 4, 6 and 8 hours following a fat-rich meal in 29 women with PCOS (NIH-criteria) and 33 normal women. Assay specificity was determined by the addition of varying concentrations of human-derived apoB100 to pooled serum. Insulin sensitivity (Si) was estimated using the frequently-sampled-intravenous glucose-tolerance-test.

Women with PCOS had greater BMI (38.29 vs. 29.77kgm^2) and lower Si (1.68 vs. 4.24) compared to controls ($P< 0.001$). At all time-points, triglyceride levels were greater in PCOS ($p=0.01$) and correlated closely with ApoB48 levels ($r=0.52$-0.77, $p< 0.001$). ApoB48 levels were non-significantly greater at all time-points in the PCOS group and correlated positively ($p< 0.05$) with total and free testosterone, Si, waist/hip ratio (WHR) and percentage body fat. Multiple regression analysis revealed free testosterone, Si and WHR to be independent predictors of ApoB48 AUC, together explaining 48% of the overall variance. Hyperandrogenism in premenopausal women is associated with increased levels of intestinally-derived lipoproteins. The clinical relevance of this requires further investigation.

Memory Stick

DETERMINATION OF APOLIPOPROTEIN(APO)B-48 CONCENTRATIONS USING A NOVEL HUMAN ELISA TECHNIQUE

Anne McGowan, W. Widdowson, J. Gibney

Department of Diabetes and Endocrinology, Adelaide and Meath Hospital, Incorporating the National Children's Hospital, Dublin, Ireland

Postprandial hypertriglyceridaemia is an independent risk factor for cardiovascular disease and postprandial triglyceride concentrations may be a better predictor of cardiovascular risk than fasting levels. The contribution of intestinally derived, triglyceride rich-chlyomicrons to the postprandial triglyceride response has not fully been described, partly due to lack of availability of a standardised method for quantification of apolipoprotein(apo)48.

We used a novel ELISA method to measure serum apoB48 levels. Assay specificity was determined by the addition of varying concentrations of human-derived apolipoprotein(apo)B100 to pooled serum. Inter-assay and intra-assay coefficient of variation was calculated using pooled serum. Average inter-assay imprecision was 11%. The assay did not detect human apoB100. Different biochemical variables were measured throughout the study to identify potential factors influencing the assay.

Plasma triglycerides and apoB48 were measured before and 2, 4, 6 and 8 hours following a mixed-meal in 44 subjects: 21 males and 23 females, average age 49 years.

Fasting apoB48 levels correlated closely with fasting triglyceride level ($r= 0.59$, $p< 0.001$). Fasting apoB48 levels also correlated with 2 hour postprandial glucose concentration ($r=0.34$, $p=0.02$).

Postprandially, apoB48 levels positively correlated with postprandial triglyceride concentrations at all time points($r=0.59$-0.7, $p=< 0.001$).

Gender, body mass index, waist/hip ratio (WHR) and insulin resistance (calculated by HOMA IR) had no significant effect on apoB48 concentration.

Free T4 concentration inversely correlated and serum TSH levels positively correlated with apoB48 AUC ($r= -0.34$, $p=0.02$ and $r=0.30$, $P=0.04$ respectively).

This suggests that thyroid function is associated with increased levels of intestinally-derived apolipoprotein and requires further investigation.

Memory Stick

EFFECTIVENESS OF OMACOR MONOTHERAPY IN SEVERE HYPERTRIGLYCERIDEMIA

Dimitris Agapakis[1], M. Papagianni[1], N. Katsiki[2], K. Tziomalos[2], C. Savopoulos[2], A. Satsoglou[1], A. Hatzitolios[2]

[1]Department of Internal Medicine, General Hospital of Goumenissa, Kilkis, [2]1st Propedeutic Department of Internal Medicine, AHEPA Hospital, Aristotle University of Thessaloniki, Thessaloníki, Greece

Background: Extremely high triglyceride (TG) concentrations are associated with several diseases, including coronary heart disease and acute pancreatitis. Current guidelines recommend that TG levels should be < 150 mg/dl. Fibrates represent first-line treatment for hypertriglyceridemia, but are implicated in the induction of serious side effects. Omega-3 fatty acids are also well-known hypotriglyceridemic agents with proven effectiveness particularly in mild forms of the disorder. The aim of our study was to examine the effectiveness of Omacor (omega-3-acid ethyl esters) as monotherapy in cases of severe hypertriglyceridemia.

Methods: A total of 27 patients (19 females, mean age 53 years (27-69 years)] with primary hypertriglyceridemia (TG> 500 mg/dl) were included in the study. All patients were on hypocaloric diet and received Omacor 4g/day for 8 weeks. Lipid parameters were measured.

Results: Initial average level of TG was 675 mg/dl (530-1350 mg/dl). Omacor therapy significantly reduced TG by 29% (P< 0.001), total-cholesterol by 9% (P< 0.05) and low-density lipoprotein cholesterol by 17% (P< 0.05), whereas increased high-density lipoprotein cholesterol by 11% (P< 0.05). However none of the patients achieved target-levels for TG and mean level of TG after the treatment was 375 mg/dl, i.e. more than double from the required target-level. The highest TG reduction reached the value of 265 mg/dl.

Conclusions: Omacor monotherpay was associated with a significant reduction in TG concentrations but failed to achieve the recommended target-levels. Therefore, in severe hypertriglyceridemia, omacor therapy should be considered only as a combination treatment to enhance its effectiveness.

Memory Stick

A CORRELATION BETWEEN HYPERLIPIDEMIA, HYPERGLYCEMIA AND TESTICULAR FUNCTIONS IN ALBINO RATS: A TESTICULAR POPULATION DYNAMICS

Ashok Purohit

Zoology Deparment, JNV University, Jodhpur, India

To evaluate the correlation of severe hyperlipidemia and hyperglycemia on testicular functions in albino rats. Male albino rats were fed with high cholesterol diet with the dose of 1000mg/kg body weight for one month. Similarly diabetes was induced to male albino rats by injecting streptozotocin at the single dose of 75mg/kg body weight intraperitonial in citrate buffer followed by feeding saturated glucose solution at regular intervail. The treatment altered the testicular cell population dynamics and the sperm motility and density in cauda epididymis and testis. Cholesterol treated animal showed severe degeneration in testicular cell population with reductions in the number of the spermatogonia, primary and secondary spermatocytes and spermatids to the extents of 58.9%,75.3%.91.7% and 99%. However, in diabetic rats only primary spermatocytes and spermatids number were reduced to 58% and 70%. In both the cases i.e hyperlipidemic and diabetic the number of degenerating Leydig cells number were significantly increased. Sperm motility and density in cauda epididymis and sperm density in testis in both treatments were reduced significantly. It is concluded from above that the severe hyperlipidemia and hyperglycemia leads to testicular dysfunction. The quantitative testicular cell population results indicate that hyperlipidemia and hyperglycemia affected the testicular androgen level either by inhibiting Leydig cell or hypothalamus pituitary functions.

Keywords: Hyperlipidemia, Hyperglycemia, Leydig cell, Testicular population.

Memory Stick

THE WHEY FERMENTATION PRODUCT MALEABLE PROTEIN MATRIX-SL0905 DECREASES TRIGLYCERIDE CONCENTRATIONS IN PATIENTS WITH HYPERCHOLESTEROLEMIA: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

I. Gouni-Berthold[1], D.M. Schulte[1], W. Krone[1], **Heiner K. Berthold**[2]

[1]Department of Internal Medicine II, University of Cologne, Cologne, [2]Charite University Medicine Berlin, Berlin, Germany

Objective: The malleable protein matrix MPM-SL0905, a whey fermentation product, is produced by an innovative fermentation process using the lactic acid bacteria strain *Lactobacillus kefiranofaciensis* R2C2. Since evidence from animal models suggests that MPM-SL0905 decreases serum lipid concentrations, purpose of the present study was to assess the hypothesis that MPM-SL0905 exerts lipid-lowering effects in humans.

Methods: Multi-center, randomized, double-blind, placebo-controlled, parallel group trial. A total of 161 patients (50% male; age 54.5±9.8 years, BMI 26.3±3.6 kg/m^2) with hypercholesterolemia (baseline LDL-cholesterol concentrations of 181±30 mg/dL) and normal triglyceride levels (131±55 mg/dL) were randomized to receive MPM-SL0905 (2 x 15 grams per day) or matching placebo. An open-label 6 weeks diet run-in phase was followed by a double-blind 12 weeks treatment phase after randomization. The data were analyzed on an intention-to-treat basis in 95.7% of the subjects. Primary outcome measure was the percent change of LDL-cholesterol. Secondary outcome measures were changes in triglycerides and HDL-cholesterol concentrations as well as changes in other cardiovascular risk factors.

Results: Triglyceride levels were decreased by 7.9±4.7% in the MPM-SL0905 group compared to placebo (P = 0.022). LDL-cholesterol concentrations decreased by 5.3±2.6% (P = 0.0416) in the subjects treated at the university outpatient lipid clinic (47% of total patients), but only by 1.8±1.2% (n.s.) in the total cohort, compared to placebo. HDL-cholesterol and blood pressure levels remained unchanged. MPM-SL0905 was tolerated well without severe adverse events.

Conclusions: MPM-SL0905 has significant triglyceride-lowering properties in patients with hypercholesterolemia. Its effects on LDL-cholesterol concentrations deserve further investigation.

Memory Stick

INFLUENCE OF SIMVASTATIN ON APOB-100 SECRETION IN NON-OBESE SUBJECTS WITH MODERATE HYPERCHOLESTEROLEMIA: A STABLE ISOTOPE STUDY

Heiner K. Berthold[1,2], P.H.R. Barrett[3], I. Gouni-Berthold[4]

[1]Charite University Medicine Berlin, Berlin, [2]Department of Clinical Pharmacology, University of Bonn, Bonn, Germany, [3]University of Western Australia, Perth, WA, Australia, [4]Department of Internal Medicine II, University of Cologne, Cologne, Germany

Objective: 3-Hydroxy-3-methylglutaryl coenzyme A-reductase inhibitors (statins) decrease apolipoprotein B-100-containing lipoproteins by increasing their fractional catabolic rates through LDL receptor-mediated uptake. Their influence on hepatic secretion of these lipoproteins is controversial. Purpose of the present study was to examine the impact of simvastatin on the secretion of apoB-100-containing lipoproteins in fasting non-obese, moderately hypercholesterolemic human subjects.

Methods: Turnover of apoB-100-containing lipoproteins was investigated using stable isotope-labeled tracers. Multicompartmental modeling was used to derive kinetic parameters. Eight male subjects (body mass index 25 ± 3 kg/m^2) with moderate hypercholesterolemia (low density lipoprotein-cholesterol 135 ± 30 mg/dL) and normal triglycerides (111 ± 44 mg/dL) were examined under no treatment (A), under chronic treatment with simvastatin 40 mg/day (B) and after an acute-on-chronic dosage of 80 mg simvastatin under chronic simvastatin treatment (C).

Results: Lipoprotein concentrations changed as expected under 40 mg/day simvastatin. Fractional catabolic rates increased in IDL and LDL but not in VLDL fractions versus control (VLDL +35% in B [n.s.] and +21% in C [n.s.]; IDL +169% in B [*P*=0.08] and +187% in C [*P*=0.032]; LDL +87% in B [*P*=0.025] and +133% in C [*P*=0.025]). Chronic (B) and acute-on-chronic simvastatin treatment (C) did not affect lipoprotein production rates (VLDL -8% and -13%, IDL +47% and +38%, and LDL +19% and +30% in B and C, respectively [all comparisons n.s.]).

Conclusions: The data indicate that simvastatin does not influence the secretion of apoB-100-containing lipoproteins in non-obese, moderately hypercholesterolemic subjects.

Memory Stick

SERUM ALBUMIN ISOLATED FROM DIABETIC OR UREMIC RATS ENHANCES THE MOUSE PERITONEAL MACROPHAGES LDL UPTAKE

A.B. Carreiro, A. Machado-Lima, R.T. Iborra, R.S. Pinto, G. Castilho, J.C. Rocha, J.T. Machado, E.R. Nakandakare, S. Catanozi, **Marisa Passarelli**

Lipids Laboratory (LIM 10), Faculty of Medical Sciences of the University of Sao Paulo, Sao Paulo, Brazil

Objectives: Atherosclerosis is prevalent in diabetes mellitus (DM) and in chronic kidney disease (CKD) and relates to oxidation and glycoxidation of macromolecules. We analyzed the *in vivo* role of DM or CKD rat albumin on the mouse peritoneal macrophages (MPM) LDL uptake.

Methods: Plasma urea, creatinine, total cholesterol (TC), triglycerides (TG) and glucose and urinary protein excretion (UPE) were determined before and 60 days after male Wistar rats were made DM by IV streptozotocin (35mg/kg) (n = 8), or CKD by 5/6 nephrectomy (n = 8), together with control animals (C; n = 9). FPLC isolated serum albumin was purified by alcoholic extraction. Human plasma *pool* LDL was labeled with ^{3}H-cholesteryl-oleoyl ether (^{3}H-COE) and acetylated with anhydrous acetic. MPM were incubated (18 h) with albumin from C, DM or CKD (1mg/mL) followed by control or acetylated ^{3}H-COE-LDL (50µg/mL, 5 h). Plasma anti-carboxymethyllysine (anti-CML) was determined by ELISA. Comparisons among groups were done by one-way ANOVA.

Results: Body weight was reduced and plasma glucose and anti-CML increased in DM rats compared to C and CKD. TC, TG, urea, creatinine and UPE were higher in CKD compared to C and DM. Control-^{3}H-COE-LDL MPM uptake (µg cholesterol/ µg cell protein) was greater after treatment with DM (14,4$\pm$0,7) or CKD (15,0$\pm$0,7) than with C albumin (11,5$\pm$0,3; $p < 0,001$). Acetylated ^{3}H-COE-LDL uptake was greater than C-^{3}H-COE-LDL but similar among groups.

Conclusion: *In vivo* modified albumin may contribute to atherosclerosis in DM and CKD by enhancing the LDL-cholesterol MPM uptake.

Funding: FAPESP, Brazil

Memory Stick

SEX DIFFERENCES IN EFFECTS OF NPC1L1 GENE POLYMORPHISM ON CHOLESTEROL ABSORPTION

Tomomi Maeda[1], A. Honda[2], Y. Mashimo[1], Y. Takeoka[1], D. Yasuda[1], N. Yoshimura[1], J. Kusano[1], K. Tsukamoto[3], T. Ishikawa[1], M. Kinoshita[1], Y. Matsuzaki[2], T. Teramoto[1]

[1]Internal Medicine, Teikyo University School of Medicine, Tokyo, [2]Gastroenterology, Tokyo Medical University, Kasumigaura Hospital, Ibaraki, [3]Internal Medicine, University of Tokyo, Tokyo, Japan

Purpose: NPC1L1 is playing an important role in small intestinal cholesterol absorption. We previously analyzed NPC1L1 gene polymorphism in Japanese subjects and reported differences in cholesterol absorption due to the gene polymorphism. However, since there are known to be sex differences in cholesterol metabolism, we have analyzed sex differences in relationship between NPC1L1 gene polymorphism and cholesterol absorption.

Subjects and methods: We examined 113 healthy adult Japanese volunteers (58 males and 55 females) who gave written informed consent. Blood was collected in the fasting state, lathosterol (latho) was measured as a marker of cholesterol synthesis, and campesterol (campe) and sitosterol (sito) as markers of cholesterol absorption, by LC-MS/MS. Leukocyte DNA were used to determine NPC1L1 three SNPs (1735C>G, 25342A>C, and 27677T>C) were analyzed by the PCR-RFLP method.

Results: Female subjects had a significantly higher serum HDL-C level and lower LDL-C and TG levels than male subjects. The level of latho was significantly higher in males than in females. Significant negative correlations were found between latho and sito and between latho and campe in males, while no significant correlations were found in females.

Among males, the levels of sito and campe were significantly higher in the NPC1L1 genotype 1735G/G group than in the 1735G/C + C/C group, similar to the results we previously reported. Among females, no differences were found between the two groups.

Conclusion: This study suggested that cholesterol synthesis is enhanced in males compared to females and that NPC1L1 gene polymorphism was determinant of cholesterol absorption only in males.

Memory Stick

FUNCTIONALITY OF *PLA2G2A* PROMOTER VARIANTS ASSOCIATED WITH SPLA2-IIA LEVELS

Holly Exeter, J. Palmen, P.J. Talmud

University College London, London, UK

Objective: Secretory (s)PLA2-IIA has been identified as a novel CHD risk marker, and measures of sPLA2 activity and mass have been associated with acute coronary syndrome and CHD risk. We previously examined the association of tSNPs in the *PLA2G2A* gene, encoding, sPLA2-IIa, and identified a strong association of 5 variants with both mass and activity. The aim of this study was to identify if any of these SNPs could explain these associations.

Methods: The functionality of 4 promoter SNPs, rs1774131, rs11573142, rs11573156 and rs3753827, was tested by luciferase assay. A 2.4kb fragment upstream of the translation start site of the *PLA2G2A* promoter, was cloned into the pGL3basic vector and all four rare variants, in different combinations, were introduced into the fragment. Twenty-four hours after transfection cells were stimulated with IL-6 and the luciferase assay was done after 48hours. Promoter activity was normalised to pGL3basic.

Results: Compared to wild-type the construct with all 4 rare variants increased promoter activity by 30%. When examined individually rs1774131 increased promoter activity 3 fold while rs11573156 increased activity by 50%. rs11573142 decreased activity to almost undetectable levels, while rs3753827 showed no difference.

Conclusions: These results confirm rs11573156 (763C>G), which is associated with a 60% raising effect on sPLA2 mass, to be functional. Whether these SNPs act co-ordinately to affect function will be determined by examination of constructs bearing different combination of variants.

Memory Stick

RELATIONSHIPS BETWEEN LDL-C, APOB AND NON-HDL-C WITH HSCRP IN HYPERCHOLESTEROLEMIC PATIENTS AT RISK FOR CARDIOVASCULAR DISEASE

J. O'Keefe[1], M. Abuannadi[1], J. Lin[2], A. Shah[2], D. Neff[3], **Joanne E. Tomassini**[3], A. Tershakovec[3]

[1]St. Luke's Mid-America Heart Institute/University of Missouri-Kansas City, Kansas City, MO, [2]Merck & Co., Inc., Rahway, NJ, [3]Merck & Co., Inc, North Wales, PA, USA

Background: Lipid-lowering therapy (LLT) reduces both LDL-C and hsCRP levels. Associations between changes in LDL-C and hsCRP with LLT are generally small. The relationships between hsCRP and non-HDL-C and ApoB, risk factors also altered by LLT, are not well-established.

Methods: Pooled data from several studies examining the effects of statins(S) and ezetimibe+statins(E+S) in hypercholesterolemic patients were used to identify LDL-C, non-HDL-C and ApoB correlations with hsCRP. Results were classified according to whether patients were on-LLT or off-LLT upon enrollment.

Results: Correlations between LDL-C, non-HDL-C and ApoB, and hsCRP were weak at baseline and improved slightly at study-end. The table lists ApoB and hsCRP results for individuals off-LLT. Similar results are noted for non-HDL-C and LDL-C. Correlations for E+S and S were generally similar, but higher for individuals off-LLT than those on-LLT at enrollment. In multivariable modeling, several demographic, metabolic and lipid baseline factors were significantly related to hsCRP at baseline and study-end, while few covariates were significant for change from baseline.

Conclusion: Similar to LDL-C, non-HDL-C and ApoB were weakly correlated with hsCRP at baseline. Correlations improved after LLT but were still weak with very similar results noted with S and E+S therapies.

		Statin alone		Ezetimibe + Statin	
Correlation*†‡§	Association between Levels at Baseline n=9014	Association between Levels at Study End n=4579	Association between % Changes at Study End n=4579	Association between levels at Study End n=4425	Association between % Changes at Study End n=4425
Simple	0.06	0.16	0.16	0.16	0.14
Partial	0.08	0.17	0.14	0.15	0.14
Multiple	0.41	0.41	0.16	0.43	0.16

* individuals not on LLT at time of enrollment in the studies; †Spearman correlation coefficients, all p<0.0001; ‡n´s are for partial and multiple correlations; §Simple = association between 2 variables; partial = association of 2 variables while controlling for various factors (gender, race, age, diabetes, metabolic syndrome, BMI [kg/m2], baseline TG [<200 vs. ≥200 mg/dL], baseline LDL-C, baseline ApoB, baseline non-HDL-C) ; multiple = multivariable determination of variables and factors as above

[Relationship between ApoB and hsCRP off-LLT]

Memory Stick

THE ROLE OF ACYLATION STIMULATING PROTEIN IN GENESIS OF DYSLIPIDEMIAS AND IN REGULATION OF TRIACYLGLYCEROL SYNTHESIS: CLINICAL AND EXPERIMENTAL DATA

Elena Ageeva, Y. Frolova, E. Dizhe, A. Vinogradov, A. Denisenko

Institute of Experimental Medicine RAMS, Saint Petersburg, Russia

Objectives: We examined whether concentration of triglycerides (TG) in plasma depends on the level of acylation stimulating protein (ASP)/C3adesArg. We studied the influence of ASP on triacylglycerol synthesis in fibroblasts.

Methods: 70 patients were included in research (19 patients with combined hyperlipidemia (HLP), 20 patients with hypertriglyceridemia (HTG), 31 patients-control group). 5 normal subjects and 5 patients with HTG were studied for their response to oral fat load. We assessed clinical and laboratory data. We studied ASP concentration in plasma. The lipid meal consisted 200ml of 20% cream mixed with 2 eggs. In experiments in vitro ASP was added to human and mouse fibroblasts in the presence 100 mkM [3 H] oleate. The triacylglycerol synthesis was determined over a concentration range of ASP (10-30 mkg/ml).

Results: The subjects with combined HLP and HTG had increased TG level (3.21±0.81 mmol/l, $P< 0.05$; 3.94±3.16 mmol/l, $P< 0.05$ v.s. 1.57±0.41 mmol/l). There was no difference of ASP concentration between groups. Patients with c-DLP had increased total cholesterol (TC) level (7.48±0.94 mmol/l, $P< 0.05$; v.s. 5.26±0.82 mmol/l). There was no correlation between ASP and TG, TC, body mass index in all groups. After oral fat load the plasma triglyceride increased, but ASP level didn't change significantly. In experiments in vitro ASP induced increase in triacylglycerol synthesis (14-36%, $P< 0.05$).

Conclusions: ASP stimulates triacylglycerol synthesis in fibroblasts. But there is no difference in ASP level between groups of patients. We suppose, the further researches will explain the role of ASP in metabolism of TG.

Memory Stick

C-REACTIVE PROTEIN LEVEL AND CAROTID ARTERIES ATHEROSCLEROSIS IN ABDOMINAL OBESITY PATIENTS

Olga Belyaeva[1], T. Karonova[1], E. Bazhenova[1], E. Baranova[1], O. Berkovich[1], A. Berezina[2], N. Ananieva[3], V. Mandal[3], E. Shlyakhto[1]

[1]Saint-Petersburg State Medical University n.a.I.P.Pavlov, [2]Federal Centre of Heart, Blood and Endocrinology n.a.V.A.Almazov, [3]Saint-Petersburg Medical Institute n.a. V.M.Bechterev, Saint Petersburg, Russia

Objective: To assess the relationship of C-reactive protein (CRP) level and carotid arteries intima-media complex in the patients with abdominal obesity.

Methods: We evaluated severity of atherosclerosis in 203 patients (30 to 55 years of age) with abdominal obesity (according to IDF criteria, 2005). The mean age was 45.9±0.5 years, waist circumference (WC) in men - 107.8±1.3 sm and in women - 98.7±0.9 sm. We used duplex scanning ALOKA SSD-3500 (Russia) of common carotid arteries and measurement of serum lipids and CRP levels by standard methodology.

Results: Mean intima-media complex was 1.28±0.42 mm with no significant difference between genders. Atherosclerotic plaques in common or/and internal carotid artery were revealed in 39,8%. The mean CRP level was 7.31±0.66 mg/L. It was found that WC correlated with early development of atherosclerosis in carotid arteries (r=0.16, p=0,009) as well as with the level of CRP (r=0.32, p=0.0001). We did not find correlations of atherosclerosis in carotid arteries with BMI. Positive correlations were revealed between CRP and intima-media complex thickness (r=0.16, p=0.02). Negative correlation was found between CRP and triglycerides serum level (r=0.19, p=0.0003).

Conclusion: We recommend test of duplex scanning of carotid arteries in patients with abdominal obesity older than 30 years for evaluation of early signs of atherosclerosis.

Memory Stick

FUNCTIONAL ANALYSIS OF LDLR PROMOTER MUTATIONS ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA

Isabel De Castro-Orós[1], L. Palacios[2], S. Pampín[3], N. Plana[4], L. Masana[4], M. Stef[2], F. Civeira[5], J.C. Ródriguez-Rey[3], M. Pocoví[1]

[1]Dpto. Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza. Instituto Aragonés de Ciencias de la Salud (I+CS), Zaragoza, [2]Progenika Biopharma, S.A., Derio, Vizcaya, [3]Dpto. Biología Molecular, Universidad de Cantabria, Santander, [4]Unitat de Medicina Vascular i Metabolisme, Universitat Rovira i Virgili, CIBERDEM, Reus, [5]Unidad de Lípidos. Laboratorio de Investigación Molecular, Hospital Universitario Miguel Servet, Zaragoza, Spain

Introduction: Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in LDLR gene affecting the protein functionality. Several mutations have been described in the response element within LDLR promoter, and associated with FH phenotype.

Material and methods: The analysis by Lipochip® platform (Progenika Biopharma) for searching PCSK9, APOB and LDLR mutations showed that 4 patients affected of FH, were carriers as heterozygous of non-described mutations in LDLR promoter: c.(-208)A>T, c.(-140)C>T, c.(-136)C>G and c.(-155)_(-150)delACCCC(-155)_(-154)insTTCTGCAAACTCCTCCC.

In silico assays with MatInspector (Genomatix) and Electrophoretic Mobility Assays (EMSA) were accomplished to determine the molecular effect of these mutations in LDLR transcriptional activity. Furthermore, a fragment of 278 bp from RLDL promoter, from -227 to +51, was amplified from DNA of individuals with the subsequent mutations in heterozygosis. Each PCR product, carrier of one mutation, was cloned into a luciferase reporter gene vector pGL3-Basic and transfected into HepG2 using Lipofectamine 2000™ (Invitrogen). Luciferase assays were performed using Dual-Luciferase® Reporter Assay (Promega) in triplicate.

Results: *In silico* and EMSA experiments showed a lower transcription factor binding when c.(-140)C>T, c.(-136)C>G, and c.(-155)_(-150)delACCCC(-155)_(-154)insTTCTGCAAACTCCTCCC mutations were present. Moreover, luciferase assays prove a significant reduction of 94%, 95% and 78%, respectively in LDLR transcriptional activity. These mutations are located in the response elements named as repeat 1 and 2 in the LDLR promoter. No significant differences were observed for the c.(-208)A>T mutation.

Conclusion: It is important to analyze the functionality for mutations being in LDLR promoter to perform a correct diagnosis of FH.

Memory Stick

NATIVE AND GLYCOXIDIZED HIGH DENSITY LIPOPROTEIN (HDL) MODULATE ADRENAL STEROIDOGENESIS VIA SCAVENGER RECEPTOR CLASS B, TYPE I (SR-BI)

Steffi Kopprasch[1], S. Saha[1], J. Graessler[1], J. Pietzsch[2], S.R. Bornstein[1]

[1]Department of Internal Medicine 3, C.G.Carus Medical School, University of Technology Dresden, [2]Department of Radiopharmaceutical Biology, Institute of Radiopharmacy, Research Center Dresden-Rossendorf, Dresden, Germany

Objective: Overactivity of the renin-angiotensin-aldosterone system (RAAS) has been causally related to the pathogenesis of type 2 diabetes and its adverse cardiovascular consequences. Since HDL-derived cholesteryl ester serves as a major source of adrenal hormone synthesis and, moreover, systemic high glucose level may affect lipoprotein structure and function, we investigated the effect of diabetic HDL modification on adrenocortical hormone synthesis.

Methods: HDL isolated from blood of healthy volunteers were glycoxidized for 6 days in the presence of 200 mmoL/L glucose. Human adrenocortical cells (H295R) were cultured in DMEM/F12 medium and incubated with native (natHDL) or modified HDL (glycoxHDL). Aldosterone, released in the medium, was measured by RIA. Adrenal SR-BI expression was determined by quantitative RT-PCR and densitometric analysis of Western blotting.

Results: HDL glycoxidation resulted in a 6.4-fold increase of TBARS content ($p < 0.001$) with only minor changes of the protein moiety as estimated by protein carbonyl levels and relative fluorescence (365/430 nm). Incubation of H295R cells with both natHDL and glycoxHDL (1 to 100 µg/mL) was followed by a significant dose-dependent enhancement of aldosterone secretion. GlycoxHDL-induced aldosterone release was blocked to 50% by the highly specific SR-BI inhibitor BLT-1 ($p < 0.001$). Native and modified HDL induced an increase in adrenocortical SR-BI mRNA and protein expression levels with glycoxHDL having greater impact.

Conclusion: HDL-mediated increase in adrenal aldosterone production is at least partially mediated by SR-BI.

Memory Stick

COPPER AND MYELOPEROXIDASE-MODIFIED LDLS ACTIVATE NRF2 THROUGH DIFFERENT PATHWAYS OF ROS PRODUCTION IN MACROPHAGES

Damien Calay[1], A. Rousseau[2], L. Mattart[1], V. Nuyens[2], P. Van Antwerpen[3], N. Moguilevsky[4], K. Zouaoui Boudjeltia[2], M. Raes[1]

[1]Research Unit in Cellular Biology (URBC), University of Namur (FUNDP), Namur, [2]Experimental Medicine Laboratory, ULB, CHU Charleroi, Montigny-le-Tilleul, [3]Laboratory of Pharmaceutical Chemistry, Universite Libre de Bruxelles, Brussel, [4]Technology Transfer Office, University of Namur (FUNDP), Namur, Belgium

Low-density lipoprotein (LDL) oxidation is a key step in atherogenesis, promoting the formation of lipid-laden macrophages. Here, we compared the effects of copper-oxidized LDLs (OxLDLs) and of the more physiologically relevant myeloperoxidase-oxidized LDLs (MoxLDLs) in murine RAW264.7 macrophages.

Both oxidized LDLs, contrary to native LDLs, induced foam cell formation and an intracellular accumulation of reactive oxygen species (ROS). This oxidative stress was responsible for the activation of the NF-E2-related factor 2 (Nrf2) transcription factor, and the subsequent Nrf2-dependent overexpression of the antioxidant genes, *Gclm* and *HO-1*, as evidenced by the invalidation of Nrf2. Interestingly, MoxLDLs always induced a stronger response than OxLDLs. These differences could be partly explained by specific ROS-producing mechanisms differing between OxLDLs and MoxLDLs. Whereas both types of oxidized LDLs caused ROS production partly by NADPH oxidase, only MoxLDLs-induced ROS production was dependent on cPLA2.

This study highlights that OxLDLs and MoxLDLs induce an oxidative stress, through distinct ROS-producing mechanisms, which is responsible for the differential activation of the Nrf2 pathway. These data clearly suggests that results obtained until now with copper oxidized-LDLs should be carefully reevaluated taking into consideration physiologically more relevant oxidized LDLs.

Memory Stick

VOLUNTARY WHEEL RUNNING BENEFICIALLY AFFECTS CHOLESTEROL TURNOVER AND ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC MICE

Maxi Meissner[1], E. Lombardo[2], T.H. van Dijk[3], R. Havinga[1], U.J.F. Tietge[1], R. Boverhof[1], T. Boer[3], K. Bijsterveld[3], F. Kuipers[1,3], A.K. Groen[1,3]

[1]Laboratory of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, Groningen, [2]Department of Medical Biochemistry, Academic Medical Center Amsterdam, Amsterdam, [3]Department of Laboratory Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands

Objective: Regular exercise reduces cardiovascular risk in humans by reducing cholesterol levels but underlying mechanisms are not fully understood. We questioned whether voluntary wheel running provokes specific modulations in cholesterol turnover that translate into reduced cardiovascular risk in hypercholesterolemic mice.

Methods: Male Ldlr deficient mice (8 wk old) had either access to a voluntary running wheel for 12 weeks (RUN) or remained sedentary (CONTROL), both groups were fed a high cholesterol diet. Running activity and food intake were recorded every other day. Fractional cholesterol absorption was measured utilizing the dual isotope technique. At 12 weeks of intervention, feces, bile and plasma were collected to determine fecal, biliary and plasma parameters of cholesterol metabolism. Atherosclerotic lesion size was determined in the aortic arch.

Results: RUN were lighter (p=0.002), food consumption increased by ~18% (p=0.004), yet, plasma cholesterol levels decreased by ~12% (p=0.035) and plasma lipoprotein profiles improved compared to CONTROL. Aortic lesion size decreased by ~43% (p=0.037). Running had no effect on fractional cholesterol absorption (p=0.84). However, running modulated cholesterol catabolism by enhancing cholesterol turnover into bile acids and increasing fecal sterol output: RUN displayed an increased biliary bile acid secretion (p=0.007) and increased fecal neutral sterol (p=0.002) and bile acid (p=0.009) outputs compared to CONTROL indicating that reverse cholesterol transport was increased in RUN.

Conclusion: Voluntary wheel running reduces cardiovascular risk in hypercholesterolemic mice. Increased cholesterol turnover and reverse cholesterol transport may underlie the beneficial effect of voluntary exercise in mice.

Memory Stick

TARGETING ONLY HIGH LDL-CHOLESTEROL DOES NOT ELIMINATE RESIDUAL CARDIOVASCULAR RISK

Marit Granér[1], O. Rimpiläinen[1], H. Tikkanen[1], M.-L. Lokki[2], M.S. Nieminen[1], M.-R. Taskinen[1], J. Sinisalo[1]

[1]Helsinki University Central Hospital, [2]Helsinki University, Helsinki, Finland

Background: Managed care initiatives to reduce cardiovascular disease (CVD) risk have, to date, focused almost exclusively on statins, which are primarily low-density lipoprotein cholesterol (LDL-C) lowering agents. However, a significant number of individuals being treated continue to experience cardiovascular events.

Methods: Concentrations of lipids, lipoproteins, oxidized LDL (oxLDL), and high-sensitivity CRP (hs-CRP) were measured in a cohort of 726 patients without diabetes mellitus (mean age 65.5 ± 11.2; 437 men, 289 women) assigned to coronary angiogram. Based on clinical and angiographic results subjects were divided into three groups: acute coronary syndrome (ACS) (n=242), stable angina pectoris (SAP) (n=242), and normal coronary artery (NCA) (n=242).

Results: LDL-C (P=0.002), oxLDL (P=0.043), and hs-CRP (P< 0.001) were higher in the ACS group than in the SAP group, but were insignificant between SAP and NCA groups. Triglyceride (TG) and apolipoprotein B (apoB) levels did not differ between the study groups. HDL-cholesterol (HDL-C) and apolipoprotein A1 (apoA1) concentrations were lower in the ACS (P< 0.001) compared with the SAP group (P< 0.001). Similarly, HDL-C and apoA1 were lower in the SAP group (P< 0.001) than in the NCA group (P< 0.001).

Conclusions: Even after treatment with statins there is a significant residual risk for cardiovascular events. Our study shows that this risk is predominantly due to low HDL-C and oxidative stress.

Memory Stick

EFFICACY AND TOLERABILITY OF SUSTAINED RELEASE FORMULATION OF SIMVASTATIN; IS THERE ANY DIFFERENCE BETWEEN MORNING TIME OR NIGHTTIME INTAKE GROUP?

Sang-Hyun Kim[1], M.S. Hyeon[2], G.R. Han[3], S.W. Cho[4], Y.G. Kim[5], H.S. Seo[6], S.H. Park[7]

[1]Cardiology, Internal Medicine, Seoul National University Boramae Hospital, [2]Cardiology, Internal Medicine, Sooncheonhyang University Hospital, [3]Cardiology, Internal Medicine, Hallym University Medical College, Seoul, [4]Cardiology, Internal Medicine, Chesaeng Hospital, Seongnam, [5]Cardiology, Internal Medicine, Dong Gook University Hospital, Goyang, [6]Cardiology, Internal Medicine, Korea University Guro Hosptial, [7]Cardiology, Internal Medicine, Ehwa University Hospital, Seoul, Republic of Korea

B**ackground:** Statins with short duration of action were recommended to nighttime intake for maximal lipid-lowering effect. But nighttime intake resulted in lower compliance. This study was designed to evaluate the efficacy on lipoprotein profile and tolerability between morning and night intake of newly developed sustained release formulation of simvastatin in hypercholesterolemic patients.

Methods: This was prospective randomized, double blind, multicenter trial at 5 centers. Patients who were candidates of drug therapy with low density lipoprotein cholesterol (LDL-C) between 130 and 220 mg/dL and triglyceride < 400 mg/dL were enrolled. These patients were randomized into morning or night group, and received sustained release form of simvastatin 20 mg and placebo twice per day for 8 weeks.

Results: 298 patients were screened, 132 patients were enrolled in this study (64 for morning group, 68 for night group). LDL-C level was decreased significantly both in morning and night intake group and there was no significant between-group differences (-35.7% vs -38.5%, p=0.26). There were no significant differences in changes of total cholesterol, triglyceride and HDL-C level between two groups (-26.2% vs -27.6 %, -12.4% vs -7.3% and +10.5% vs +11.8%, p=NS). There were no significant differences in changes of apoB/A1, LDL/HDL ratio and target achievement rates between two groups. Drug compliance in the morning was significantly greater than that of night in two groups.

Conclusion: Morning intake of sustained release form of simvastatin improved lipoprotein profiles significantly and, there was no difference between morning and night intake. Morning intake significantly improved drug compliance.

Memory Stick

LDL-ASSOCIATED LYSOZYME AND APOJ - SPECIFIC DISTRIBUTION IN INDIVIDUALS WITH TYPE 2 DIABETES AND THE METABOLIC SYNDROME

Camilla Pettersson[1], H. Karlsson[2], B. Fagerberg[1], M. Ståhlman[1], T. Larsson[1], M. Lindahl[2], J. Borén[1], O. Wiklund[1], L. Fogelstrand[1]

[1]Wallenberg Laboratory for Cardiovascular Research, Department of Molecular and Clinical Medicine, Institute of Medicine, Gothenburg, [2]Division of Occupational and Environmental Medicine, Department of Molecular and Clinical Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

Individuals with type 2 diabetes (T2D) and the metabolic syndrome display an atherogenic lipoprotein profile, including small dense LDL with decreased cholesterol content and increased apoCIII content. The aims of this study were to examine the general protein composition of LDL in an explorative manner, and to compare the protein composition of LDL from individuals with T2D and the metabolic syndrome (T2DM) with that of LDL from healthy controls.

LDL from 61-year-old men was isolated using size exclusion chromatography or deuterium oxide-based ultracentrifugation. LDL-associated proteins were identified using mass spectrometry and quantified using two-dimensional gel electrophoresis or ELISA.

LDL from individuals with T2DM displayed a different protein composition than LDL from control individuals, including increased contents of apoJ and lysozyme, and decreased apoA1. ApoJ and lysozyme correlated directly with apoCIII content and inversely with cholesterol content in LDL, indicating the presence of these proteins preferably on small dense LDL. In serum of individuals with T2DM, lysozyme levels, but not apoJ levels, were increased. *In vitro*, lysozyme associated more with advanced glycated LDL (AGE-LDL) than with unmodified LDL.

Since small dense LDL is more easily glycated than normal LDL, we propose that the enrichment of lysozyme in LDL from individuals with T2D might be due to its ability to bind to AGE moieties. Since both lysozyme and apoJ have been shown to be antioxidative and atheroprotective in cell- and mouse studies, the association of these proteins with diabetic LDL might be protective against oxidative stress induced by modifications in LDL.

Memory Stick

CHOLESTERYL ESTER STORAGE DISEASE (ACID LIPASE DEFICIENCY): ANOTHER FACTOR FOR EARLY ATHEROSCLEROSIS DISEASE. EXPERIENCE FROM THE GREEK POPULATION

Euridiki Drogari[1], P. Progias[1], E. Koniari[1], N. Manolaki[2], H. Christomanou[3]

[1]1st Department of Pediatrics, Choremio Research Laboratory, Unit of Metabolic Diseases, Aghia Sophia Children's Hospital, University of Athens, Medical School, [2]2nd Department of Pediatrics, Aghia Sophia Children's Hospital, [3]Research Institute for Lysosomal and Neurodegenerative Disorders, Athens, Greece

Cholesteryl ester storage disease (CESD) is due to acid lipase deficiency with autosomal recessive inheritance. We describe our experience from 17 families with CESD. All families were screened for three consecutive generations. They originate from different areas of Greece and the parents are unrelated. The families were identified after referral to the Lipid Clinic for high lipid levels and liver disease. Lipid levels (total cholesterol, triglycerides, HDL, VLDL, LDL), blood and liver tissue enzymatic activity from 19 children-patients, 34 parents, 20 siblings, 24 grandparents and 32 uncles/aunts were screened. All 19 patients had liver biopsies and 10 of them had skin biopsies where enzyme activity was measured. Liver CT scan showed fibrotic fatty liver and calcificated areas in all patients. Histochemistry in liver biopsy showed adipose of cholesterol ester with fibrosis. Total cholesterol levels ranged from 280-450mg/dl, triglycerides from 420-1100mg/dl, HDL from 19-38mg/dl, and LDL from 180-395mg/dl in the homozygotes. Hepatic enzymes were elevated 4 to 10-fold compared to normal. Enzyme activity in blood and liver fibroblasts was 0-2% of control values for homozygotes and 35-48% for heterozygotes. Increased lipid levels and early ischemic heart disease was reported in 72% of adult family members. Patients were homozygotes and compound heterozygotes (5 mutations identified in 17 patients). Treatment started after diagnosis using low fat diet, statins and Ezetimibe. There was considerable improvement of lipid levels and liver enzymes (40-90% reduction) during a follow-up of 1-20 years. CESD contributes to premature atherosclerosis in homozygote and heterozygote patients and should be further investigated.

Memory Stick

PROTHROMBOTIC MARKERS IN DYSLIPIDEMIC PATIENTS

D. Karasek, **Helena Vaverkova**, M. Halenka, D. Jackuliakova, Z. Frysak, L. Slavik, D. Novotny

University Hospital Olomouc, Olomouc, Czech Republic

Introduction: We evaluate the plasma levels of prothrombotic markers - von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (t-PA) - in subjects with dyslipidemia. We also investigated the association between these markers and selected risk factors for atherosclerosis and markers of insulin resistance.

Methods: 234 patients with dyslipidemia and their first degree relatives were assessed for lipids, prothrombotic markers and parameters of insulin resistance. Individuals were divided into four dyslipidemic phenotypes (DLP) according to apolipoprotein B (apoB) and triglycerids (TG): DLP1 (n=58, apoB< 1.2g/l and TG< 1.5mmol/l), DLP2 (n=47, apoB< 1.2g/l a TG≥1.5mmol/l), DLP3 (n=31, apoB≥1.2g/l a TG< 1.5mmol/l) a DLP4 (n=98, apoB≥1.2g/l a TG≥1.5mmol/l).

Results: After adjustment to age, sex and BMI, significant differences were observed in levels of PAI-1 between DLP1 (62.5[35.9-82.9] ng/ml) and hypertriglyceridemic phenotypes - DLP2 (82.2[61.1-122.1] ng/ml, p< 0.01) and DLP4 (91.4[63.5-111.8] ng/ml, p< 0.001). Levels of t-PA were statistically different only between DLP1 and DLP4 (1.9[0.9-3.3] ng/ml versus 5.3[2.5-8.6] ng/ml, p< 0.05). There were no significant differences in levels of vWF. By using a multiple regression model, PAI-1 and t-PA levels were predicted by markers of insulin resistance (insulin, proinsulin, HOMA, C-peptide).

Conclusions: Compared to normolipidemic subjects, individuals with hypertriglyceridemic DLP have increased levels of PAI-1, which are independently associated with insulin resistance. The increase of t-PA is presented only in those with simultaneously elevated levels of TG and of apoB. There were no significant differences in levels of vWF between dyslipidemic and normolipidemic individuals.

Supported by grant IGAMZCR NS/10284.

Memory Stick

INCREASED INSULIN RESISTANCE, INSULIN SECRETION, THROMBOTIC AND INFLAMMATORY FACTORS IN CHILDREN, ADOLESCENTS AND YOUTH WITH METABOLIC SYNDROME

Vesna Dimitrijevic-Sreckovic[1], P. Djordjevic[1], S. Damjanovic[1], B. Sreckovic[2], E. Colak[3], I. Soldatovic[4], A. Stamenkovic[1]

[1]Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [2]Internal Medicine, Clinical Center Bezanijska Kosa, [3]Institute of Medical Biochemistry, [4]Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia

Aims: To analyze insulin resistance (IR), insulin secretion, thrombotic and inflammatory factors in children, adolescents and youth with metabolic syndrome (MS).

Methods: The study included 185 obese individuals (age 7 to 30): I- children (7 to 15), II- adolescents (16 to 20) and III- youth (20 to 30). Three of the following five criteria were used for MS diagnosis: waist circumference (WC) > 90 Pct.; triglycerides (TG) >1.7 mmol/L; high density lipoprotein cholesterol (HDL-C) < 1.0 mmol/L; hypertension > 90 Pct.; glycemia >6.0 mmol/L.

Results: MS, increasing considerably with age, was found in 29.3% children, 40.5% adolescents and 46.7% youth. Patients with MS had increased WC: (I-88.1±13.9, II-112.4±19.9, III-108.6±19.4 cm)), blood pressure (I-120.0±14.6 / 78.5±11.1, II-128.0±9.1 / 82.1±9.9, III- 130.2±23.7 / 86.3±10.6mmHg), HOMA IR (I-6.9±3.0, II-11.5±17.3., 13.8±18.6), HOMA β (I-885.7±1206.0, II 812.0±1412.2,III-797.3±808.9), triglycerides (I-2.0±0.8, II- 2.0±0.75, III-2.6±2.4mmol/l), CRP (I-2.9±6.2, II- 7.5±8.2, III-8.4±7.4mg/l), PAI-1 (I-5.8±.7, II- 6.6±1.0,III- 6.7±1.0 U/ml) and microalbuminuria (I-34.4±25.0, II-61.9±56.5,III- 61.0±60.7mg/24h), and decreased HDL (I-0.99±0.29, II- 1.05±0.23, III-0.96±0.15mmol/l). Correlations: WC with BMI, systolic blood pressure, HOMA IR, HOMA β, basal, 30 min, 120 min and mean insulinemia, CRP ($p < 0.01$) I PAI-1 ($p < 0.05$) ; HOMA IR with HOMA β, BMI, WC, blood pressure, triglycerides, basal, 30 min, 120 min and mean insulinemia, basal glycemia ($p < 0.01$), PAI-1 ($p < 0.05$) I negatively with HDL ($p < 0.01$).

Conclusion: In MS, abdominal obesity accompanied with hyperinsulinism and insulin resistance is related to hypertension and lipid status disturbance where thrombotic and inflammatory factors and tendency to early atherosclerosis are present.

Memory Stick

USEFULNESS OF DNA ANALYSIS FOR THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA (FH) AND EXTRAORDINARILY HIGH FREQUENCY OF FH IN JAPAN

Hiroshi Mabuchi[1], A. Nohara[2], T. Noguchi[3], J. Kobayashi[3], M.-A. Kawashiri[4], H. Tada[4], M. Yamagishi[4], A. Inazu[5], J. Koizumi[6], Hokuriku FH Research Group

[1]Lipidology, Kanazawa University Graduate School of Medical Science, [2]Kanazawa University Graduate School of Medical Scienc, [3]Lipidology, [4]Cardiology, [5]Department of Laboratory Science, [6]General Internal Medicine, Kanazawa University Graduate School of Medical Scienc, Kanazawa, Japan

Objects: Familial hypercholesterolemia (FH) results from mutations in 4 “FH genes” (LDL receptor (LDL-R), apoB-100, PCSK9 and ARH protein). We analyzed DNA of FH genes in 848 heterozygous and 26 homozygous FH patients in Hokuriku district of Japan.

Methods: Clinical diagnostic criteria of hetero-FH are

1) hypercholesterolemia (TC > 225mg/dl or LDL-C > 160mg/dl) with tendon xanthoma or

2) hypercholesterolemia in 1st or 2nd degree relative of FH. Methods used for screening DNA mutations of FH are direct sequencing of PCR products, Invader Assay, MLPA and PCR-RFLP.

Results: We found 61 LDL-R gene mutants, 1 PCSK9, 1 ARH and no apoB-100 mutants. Common mutants were K790X (32.2%), IVS15-3C>A (13.5%) and FH-Tonami-2 (6.9%). A gain-of-function mutant of PCSK9 E32K was found in 47 heterozygotes (8.6%). Plasma TC levels were higher in K790X, IVS12+2T>C and IVS15-3C>A, and lower in PCSK9 E32K heterozygotes. In patients without tendon xanthoma, DNA analysis confirmed FH in 41.5%. Our DNA analysis covers only 663/850 (78%) of FH, and an advanced technology is anticipated. We found 26 homozygous FH patients in Hokuriku (3.08 millions), and the frequency of heterozygotes was calculated to be 1/172. Genotype frequencies calculated by Hardy-Weinberg distribution break down if there are consanguineous marriages. Eight homozygotes were born to consanguineous marriage, and thus the frequency of heterozygotes should be 1/207. No definite founder gene effect was found.

Conclusions: DNA analysis is an efficient and decisive method in diagnosing FH patients. The genotype frequency of FH in Japan was calculated to be at least 1/207.

Memory Stick

LEVELS OF PLASMA LIPIDS, LIPOPROTEINS AND APOLIPOPROTEINS IN OVERWEIGHT HYPERTENSIVE CHILDREN

Zorana Jelic-Ivanovic, S. Spasic, V. Spasojevic-Kalimanovska

Institute of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Objective: We examined the extent of blood lipid abnormalities in overweight and hypertensive children.

Methods: A retrospective, case-control study included 624 schoolchildren (316 boys), 7 to 13 years old. Blood lipids were measured in fasting samples.

Results: About 16% of examined children were considered overweight. Number of children with high BP was significantly higher in overweight than in children with normal weight. Overweight children with high BP had significantly higher values of TC than children with normal weight and normal BP (4.69±0.78 vs. 4.26±0.73 mmol/L) as well as TG (1.23±0.65 vs. 1.00±0.48 mmol/L), LDL-C (2.59±0.94 vs. 2.25±0.85 mmol/L) and apo-B (0.80±0.27 vs. 0.89±0.30 g/L). The values of HDL-C and apo-AI were not statistically different in the two groups.

Overweight children with high BP were 2.1 (1.5-3.0, $p< 0.001$) times more likely to have elevated level of TC than children with normal BMI . Odds ratios for other associations were: TG 2.0 (1.3-3.1, $p=0.002$), LDL-C 1.5 (1.2-2.1, $p=0.003$) and apo-B 2.4 (1.1-5.3, $p=0.03$).

Conclusions: The prevalence of overweight is increasing among youth and our results demonstrate that overweight is consistently related to several CVD risk factors. Furthermore, as adults with multiple risk factors will likely be at high risk for CVD in later life, it is of particular interest that a large proportion of these individuals are identified in early life. These findings emphasize the importance of the prevention and treatment of obesity in childhood.

Funding: This work was supported by a grant from the Ministry of Science, Republic of Serbia.

Memory Stick

CIRCULATING OXIDIZED LOW - DENSITY LIPOPROTEIN LEVELS MAY SERVE AS A RISK MARKER FOR CAROTID STENOSIS DURING STATIN THERAPY

Sofoklis Kougialis, D. Levisianou, E. Skopelitis, S. Nikolaou, A. Kroustalis, T. Gogalis, G. Groumas, A. Zervou, N. Kosmas, N. Iliopoulos, C. Mpilinis

Agios Panteleimon General Hospital, Nikea, Greece

Background and aims: Oxidized low-density lipoprotein (oxLDL) is an essential element of the atheromatous process. Statins reduce atheromatosis and cardiovascular risk, as well as circulating oxLDL. The aim of the present study was to investigate the beneficial effect of statins on circulating oxLDL and the possible correlation with changes of stenosis in patients with carotid atheromatosis.

Materials and methods: A total of 100 patients (76 males, median age 68 years) with carotid stenosis of various causes were enrolled. Those with stenosis >70% (n=50) underwent angioplasty prior to enrollment. Those with < 70% (n=50) were treated only conservatively. Both groups were given atrorvastatin in doses adequate to maintain LDL cholesterol < 100mg/dl and were followed at 1, 3, 6 and 12 months. Anthropometrics, complete lipid profile, and oxLDL were obtained in every visit. Stenosis was evaluated by ultrasonography at baseline and the end of the study.

Results: oxLDL was significantly reduced at 12 months (62.26±22.03 vs 44.49±21.75, p< 0.001). In the invasively pretreated group no significant restenosis was noticed, while in the conservatively treated group a significant reduction of stenosis was demonstrated (47.6±13.2% vs 37.7±15.7%, p< 0.001). The decrease of oxLDL correlated with the reduction of stenosis (r=0.17, p=0.018). OxLDL was an independent risk factor for restenosis in multivariate analysis (hazard ratio=4.319, p< 0.001).

Conclusion: A marked reduction of oxLDL and stenosis was shown in patients with carotid atheromatosis treated with usual doses of atorvastatin. OxLDL correlate well with and could be a useful marker of stenosis in such patients.

Memory Stick

BIOCHEMICAL CHARACTERIZATION OF LDL SUBFRACTIONS AND VERIFICATION OF SPECIFICITY OF A NEW HOMOGENEOUS ASSAY FOR SMALL, DENSE LDL-CHOLESTEROL

M. Fujimura, M. Ohta, **Yasuki Ito**

Denka Seiken Co., Ltd., Tokyo, Japan

We reported the development of a novel homogeneous assay for quantification of small dense LDL cholesterol (sd LDL-C) previously. We presented the assay design and verification results before. In this report, we present results from further characterization studies for our new assay along with biochemical features of LDL subfractions. For the development of the assay for sd LDL-C, we focused on a difference in ratios of sphingomyelin to lecithin in various LDL subfractions, and we incorporated a sphingomyelinase in the reagent to decompose Large LDL particles which are believed rich in sphingomyelin. However, there is no publication describing profile of phospholipids on various LDL particles in detail. In addition, there is no clear evidence on the reactivity of our homogeneous assay to LDL particles with different ratios of sphingomyelin to lecithin. We first obtained consecutive 21 fractions from d=1.019 to d=1.063 by ultracentrifugation. We then tested such fractions by our new assay for sd LDL-C as well as by phospholipid, sphingomyelin, triglyceride and apoB measuring reagents and also by Transmission Electron Microscopy. We got the following findings:

1. There is a clear relationship between the size of LDL and the ratio of sphingomyelin to lecithin. As the LDL particle size gets smaller, the ratio gets also smaller (25.1% in the largest particle and 8.9% in the smallest particle).
2. The reactivity of our new assay for sd LDL-C was found proportional to the ratio of sphingomyelin to lecithin and the strongest to the LDL fraction with the smallest ratio.

Memory Stick

APOLIPOPROTEIN B BOUND TO ERYTHROCYTES: A NOVEL ANTI-ATHEROGENIC MECHANISM

S.A. Bovenberg[1], A. Westzaan[1], S. Martinez-Hervas[2], G.-J. van de Geijn[3], H. Janssen[3], T. Njo[3], E. Birnie[4], A. Rietveld[1], J.W.F. Elte[1], M. Castro Cabezas[1]

[1]Intern Medicine, Sint Franciscus Gasthuis, Rotterdam, The Netherlands, [2]Endocrinology, Hospital Clínico Universitario de Valencia, Valencia, Spain, [3]Clinical Chemistry, [4]Statistics, Sint Franciscus Gasthuis, Rotterdam, The Netherlands

Aim: Apoliprotein (apo) B containing lipoproteins are closely linked to atherogenesis. These lipoproteins are transported in plasma and also bind to blood leukocytes (ATVB2008;28:792-797). This may lead to leukocyte activation and endothelial damage. We investigated wether apoB is present on erythrocytes and if there is a relationship with atherosclerosis.

Methods: Subjects with coronary artery disease (CAD+) and without (CAD-) were investigated. Anthropometric parameters and bloodsamples were collected. Erythrocyte-bound apoB was measured by flowcytometry with a polyclonal antibody and fluorescent labelled secondary antibody. Intima media thickness (IMT) measurements were carried out using B-mode ultrasound at three different projections of the far wall of each carotid artery, using the mean of these measurements.

Results: 111 Subjects were included (36 CAD+ and 75 CAD-). In all but nine subjects (4 CAD- and 5 CAD+) apoB was detected on freshly isolated erythrocytes (range from 0 to 5.5 au; mean±SEM 0.86±0.09 au). Erythrocyte-bound apoB was lower in CAD+ (0.59 ±0.09 au; mean±SEM) compared to CAD- (1.00±0.12 au; P< 0.001). IMT was increased in CAD+ subjects (0.77±0.13mm; mean±SD) compared to CAD- (0.56±0.14 mm; P< 0.001). A negative correlationship was found between erythrocyte bound-apoB and IMT (Spearmans rho -0.291; P=0.002). There was no association with plasma apoB (rho:0.10), but negative associations were found with glucose (rho:-0.21; P=0.03), diastolic blood pressure (rho:-0.21; P=0.03) and positive associations with HDL-C (rho:0.20; P=0.04) and apoAI (rho:0.20; P:0.03).

Conclusion: These data suggest a correlation between erythrocyte-bound apoB and favourable metabolic conditions. High apoB bound to erythrocytes may be protective against atherosclerosis.

Memory Stick

HNE-INDUCED 5-LOX EXPRESSION IS TRANSCRIPTIONAL REGULATED BY NF-KB/ERK AND SP1/P38 MAPK PATHWAYS VIA EGF RECEPTOR IN MURINE MACROPHAGES

S.-J. Lee, C.-E. Kim, K.-W. Seo, **Chi-Dae Kim**

Pharmacology, Pusan National University, Yangsan, Republic of Korea

5-LO is suggested as a modulator of atherosclerotic plaque instability, and co-exist with 4-hydroxynonenal (HNE) in macrophages in atherosclerotic lesions. To determine the potential role for HNE on the regulation of 5-LO expression, the promoter activity of 5-LO was evaluated in HNE-stimulated macrophages. In the present study, a genomic sequence of promoter 2.0 kb upstream of the transcription initiation site of mouse genomic DNA was amplified by PCR, and then the constructs containing a series of sequentially deleted fragments was fused to pGL3 vector containing a luciferase reporter gene. Luciferase assay showed that promoter region 1-213 bp upstream of transcription start site was responsible for HNE-enhanced transcriptional activity of 5-LO. A site-directed mutagenesis of this region revealed that transcription factors including Sp1 and NF-κB were associated with up-regulation of HNE-induced 5-LO transcription. Moreover, the role of Sp1 and NF-κB in HNE-induced 5-LO expression was confirmed by siRNA knockdown of Sp1 and NF-kB. Among the MAPK pathways, HNE-enhanced NF-κB and Sp1 activity was attenuated by an ERK inhibitor, PD98059 and a p38 MAPK inhibitor, SB203580, respectively. In addition, the receptor tyrosine kinases antagonists including AG1478, epidermal growth factor receptor (EGFR) antagonist, but not AG1295, platelet derived growth factor receptor (PDGFR) antagonist, were significantly inhibited HNE-enhanced phosphorylation of ERK and p38 MAPK. Collectively, these data suggest that 5-LO expression by HNE is transcriptional regulated via EGF receptor-mediated activation of Sp1/p38 MAPK and NF-kB/ERK pathways in murine macrophages, consequently provide novel options for therapeutic interventions to regulate 5-LO expression in atherosclerosis.

Memory Stick

SMALL, DENSE HDL3 PARTICLES EXHIBIT DEFECTIVE ANTIOXIDATIVE AND ANTIINFLAMMATORY FUNCTION IN FAMILIAL HYPERCHOLESTEROLEMIA: PARTIAL CORRECTION BY LDL-APHERESIS

H. Hussein[1,2,3], S. Saheb[3], M. Couturier[1,4], M. Atassi[3], A. Orsoni[1,2,3], A. Carrié[1,2,3], P. Therond[4], S. Chantepie[1,2,3], P. Robillard[1,2,3], E. Bruckert[3], M.J. Chapman[1,2,3], **Anatol Kontush**[1,2,3]

[1]INSERM Unit 939, [2]Université Pierre et Marie Curie-Paris 6, [3]AP-HP, Groupe Hospitalier Pitié-Salpétrière, [4]Department of Biochemistry, University Paris Descartes, Paris, France

Background: Familial hypercholesterolemia (FH) features elevated oxidative stress and accelerated atherosclerosis driven by elevated levels of atherogenic lipoproteins relative to subnormal levels of atheroprotective high-density lipoprotein (HDL). Small, dense HDL3 potently protect low-density lipoprotein (LDL) against proinflammatory oxidative damage. Whether antiinflammatory/antioxidative activities of HDL are defective in FH, and whether such defects are corrected by LDL-apheresis are indeterminate.

Methods and Results: Pre-LDL-apheresis, FH patients (n=10) exhibited elevated systemic oxidative stress (as plasma 8-isoprostanes; 3.3-fold, $p< 0.001$) vs. sex- and age-matched normolipidemic controls (n=10). Both antioxidative activity (as protection of reference LDL from oxidation) and antiinflammatory activity (as capacity to prevent accumulation of proinflammatory oxidised lipids) of HDL3 were impaired (up to -91%, $p< 0.01$) in FH. Sphingomyelin and saturated fatty acid contents were elevated in FH HDL3, resulting in enhanced lipid surface rigidity (using a fluorescent probe). The surface lipid content (phospholipids, free cholesterol) was reduced in FH (up to -15%, $p< 0.001$), whereas content of core lipids (cholesteryl esters, triglycerides) was elevated (up to +17%, $p< 0.001$). Molar apolipoprotein A-I content of HDL3 was subnormal in FH. A single LDL-apheresis session partially corrected (by up to 76%) deficient HDL antiatherogenic activities, attenuated systemic oxidative stress and partially normalised both the lipidome and surface rigidity of HDL particles.

Conclusions: FH features elevated oxidative stress and deficient antioxidative and antiinflammatory activities of small, dense HDL3; such functional deficiency is intimately linked to anomalies in lipidome and proteome, which may impair the capacity of HDL to acquire and inactivate oxidised lipids.

Memory Stick

ATORVASTATIN EFFECT ON THE DISTRIBUTION OF HDL SUBFRACTIONS AND HUMAN PARAOXONASE ACTIVITY

György Paragh, M. Harangi, H.Z. Mirdamadi, A. Kassai, Z. Derdák, I. Seres

First Department of Medicine, Medical and Health Center, University of Debrecen, Debrecen, Hungary

Human serum paraoxonase-1 (PON1) protects lipoproteins against oxidation by hydrolyzing lipid peroxides in oxidized LDL, therefore it may protect against atherosclerosis. Changes in the ratio of HDL subfractions may alter the stability and the antioxidant capacity of PON1.

Objectives: The aim of our study was to examine the effect of atorvastatin treatment on the distribution of HDL subfractions, LDL size, cholesteryl ester transfer protein (CETP), lecithin:cholesterol acyltransferase (LCAT) and human serum paraoxonase-1 (PON1) activity.

Methods: Thirtythree patients with type IIa and IIb hypercholesterolemia were involved in the study. LDL sizes and HDL subfractions were determined by gradient gel electrophoresis. CETP, LCAT and PON1 activities were measured spectrophotometrically.

Results: Three months of treatment with atorvastatin 20 mg daily significantly increased the HDL3 (+8.13 %) and decreased the HDL2a and HDL2b subfractions (-1.57% and -6.55%, respectively). The mean LDL size was significantly increased (+3.29 %). The level of oxidized LDL was significantly decreased (-46.0 %). The PON1 activity was augmented by the atorvastatin treatment (+5.0 %). The CETP activity positively correlated with the HDL2b level and negatively correlated with the HDL3 and HDL2a levels.

Conclusion: Atorvastatin alters the HDL subfractions, which may improve its antiatherogenic effect via enhancement of the PON1 activity.

Memory Stick

ATHEROGENIC POTENTIAL OF SUBCLINICAL HYPOTHYROIDISM AND WAYS OF HIS CORRECTION

Ala Logvinenko, V. Romanov, O. Mitchenko, I. Chulaevskaya

Department of Dyslipidemia, Institute of Cardiology, Kiev, Ukraine

Aims: To learn optimization of treatment of lipid & carbohydrate disoders of levothyroxine replacement therapy (LRT) for women with metabolic syndrom (MS) and subclinical hypothyroidism (SH).

Methods: 143pts with MS are surveyed 3 gr:1 gr.-49 pts with MS, SH & undergoing LRT; 2 gr-46 pts with MS, SH & without LRT; 3 gr-48 pts with MS & euthyroid. The insulin resistance (IR) was diagnosed at 51% pts by increase of the Homa-index >2,77. All pts got atorvastatin 10mg and pts of a 1 gr. additionally LRT (50mg/daily). Patients were observed at the beginning & in 6 months of treatment.

Results: Pts of 1&2 gr. had authentically large of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol than in 3 gr. This pattern of lipid abnormalities were found out at 96% women with MS, SH. Increase of triglycerides, decline of high-density lipoprotein cholesterol were registered less than in 50% pts. In 6 months the women of a 1gr. the reliable decline of TC to 4,9+0,2, LDL to 2,7+0,1mmol/l was marked. For women 2 gr. was marked a tendency to the decline of TC, LDL but they exceeded a target values for pts from MS. Pts of a 1gr. was observed proceeding in the normal sensitiveness to insulin, mainly due to the decline of insulin.

Conclusions: The lipid profile of women with a MS, SH is characterized most atherogenic disoders, by the enhanceable levels of TC, LDL. Therapy of atorvastatin at 2 gr. is insufficient for achievement of a target levels of TC, LDL. Setting of the LRT+statin, optimizes efficiency of lipidlowering therapy allowing to attain the normal levels of lipids without the increase of dose of statin and to decreasing of IR.

Memory Stick

ATORVASTATIN EFFECTS ON LIPID PROFILE IN APOE/LDLR-DEFICIENT MICE

M. Slanařová[1], **Lenka Večeřová**[1], Z. Stráský[1], A. Tichá[2], R. Hyšpler[2], P. Nachtigal[1]

[1]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Biological and Medical Sciences, [2]University Hospital and Medical Faculty in Hradec Kralove, Department of Gerontology and Metabolism, Hradec Králové, Czech Republic

Statins are first-line pharmacotherapeutic agents for hypercholesterolemia treatment in humans. However, statins effects in animal models of atherosclerosis are not very consistent. Thus we wanted to evaluate whether atorvastatin possess hypolipidemic effects in ApoE/LDLR-deficient mice on either chow or cholesterol rich diet.

Two-month-old female ApoE/LDLR-deficient mice (n=32) were randomly subdivided into 4 groups. Two control groups of animals (n=8) were fed with the either cholesterol diet (0.15% cholesterol) or chow diet and in other two groups atorvastatin was added to the cholesterol-rich diet or chow diet at the dosage of 50 mg/kg per day respectively for the period of 2 months. Serum lipoprotein fractions were prepared using sodium chloride density gradient ultracentrifugation. Total concentration and lipoprotein fraction concentration of cholesterol were measured enzymatically by conventional enzymatic diagnostic kits and spectrophotometric analysis. Atorvastatin treatment significantly decreased levels of total cholesterol, VLDL and LDL only in mice fed with cholesterol-rich diet. On the contrary, the administration of atorvastatin surprisingly resulted in a significant increase in total cholesterol and VLDL in mice by fed by chow diet. Moreover, the measurement of cholesterol content in the liver showed that atorvastatin treatment decreased cholesterol content in liver in mice fed with atherogenic diet but increased cholesterol content in liver in mice fed by chow diet only.

In this study, we demonstrated both hypolipidemic and hyperlipidemic effects of atorvastatin in mice fed either cholesterol and/or chow diet suggesting that the presence of cholesterol in the diet significantly affects statins effects in ApoE/LDLR double knockout mice.

,, This work was supported Research project MZO 0017990.”

Memory Stick

MOLECULAR STUDY OF FAMILIAL HYPERCHOLESTEROLEMIA IN BELGIUM

Eric Tarantino, S. Mabille, M. Bruniaux, O. Descamps

Centre de Recherche Médicale de Jolimont, Haine Saint-Paul, Belgium

Objective: Molecular characterization of patients with a clinical diagnosis of Familial Hypercholesterolemia (FH) in Belgium.

Methods: Blood samples were collected from 1729 subjects with high LDL-C levels. DNA was isolated and promoter and the 18 exons and their intron sequences of LDLR gene were analysed by denaturing gradient gel electrophoresis (DGGE). Each sample with one or more positive exon was sequenced twice, with forward and reverse primers, with Big Dye Terminator V1.1 Cycle Sequencing Kit and a ABI 310 prism automated sequencer. APOB was analysed by Sau 96 mismatch PCR-RLFP method.

Results: The ApoB3500 mutation was found in 59 patients and 89 different mutations in LDLR were found in 677 patients, 35 of which have not been described previously. Eight patients were found homozygous (hoFH): 1 homoallelic, 5 heteroallelic hoFH and 2 compounds heFH. Two compound heterozygotes and three of our heteroallelic hoFH had only elevated cholesterol in range of heFH. No mutation could be found in 991 patients with a FH like phenotype and will be candidates for mutations in other genes, including PCSK9.

Conclusion: It was possible to identify the genetic cause of hypercholesterolaemia in 43% of patients suspected of FH. Knowledge of the molecular basis of the disease allows correct and appropriate therapeutic measures. Genetic diagnosis also allows early identification of relatives at risk, reducing their cardiovascular risk which should see their quality of life and life expectancy increased.

Memory Stick

THE INFLUENCE OF GENES ON LIPID METABOLISM IN WEST SIBERIA CAUCASIAN POPULATION

Elena Shakhtshneider, A. Allakhverdyan, I. Kulikov, V. Maksimov, A. Romashchenko, Y. Nikitin, S. Malyutina, M. Voevoda

Institute of Internal Medicine SB RAMS, Novosibirsk, Russia

Background and aims: We investigated polymorphisms of genes of lipid metabolism and their influence on a plasma lipid levels in Caucasian population of West Siberia.

Methods: The patients included in the analyses were selected based on total cholesterol (TC) level from population sample surveyed in frame of HAPIEE project (~9000 participants, aged 45-69, men 50%). Totally 100 patients with highest total cholesterol level (TC>300mg/dl) and 100 patients with lowest total cholesterol level (TC< 200mg/dl) and 100 patients with populations total cholesterol level (TC 233.6±47,7mg/dl) were included in the analyses. The differences of level TC between groups are significant. The plasma lipid levels were determined by standard enzymatic assays. All patients were of Caucasian origin. The polymorphisms of APOAI, APOB, APOCIII, APOE, LPL, USF1 genes were analyzed by standard method.

Results: The Caucasian population of West Siberia is not significantly differs from populations of Europe and North America by frequencies of alleles and genotypes. The polymorphisms of APOAI (-75 and +83), APOB (3405Glu), APOCIII (SstI), APOE (ε2, ε3, ε4), LPL (HindIII) and USF1 (C475T and C1748T) genes have been associated with higher total serum cholesterol level.

Conclusions: Polymorphism genes assay is of importance for estimation of predisposition to common diseases on population and individual level.

This work was supported by Grant of President of Russian Federation MK-2666.2009.7.

Memory Stick

PITAVASTATIN REDUCES HIGH-SENSITIVITY C-REACTIVE PROTEIN AND IMPROVES LIPID PROFILES IRRESPECTIVELY OF BODY MASS INDICES -SUBANALYSIS OF KISHIMEN MULTI-CENTER PROSPECTIVE STUDY

Noriaki Kume[1], Y. Fujioka[2], A. Taniguchi[3], K. Tanaka[4], S. Kagimoto[5], K. Hirata[6], Y. Nakamura[7], T. Yamamoto[8], S. Fujimoto[9], Y. Hamamoto[9], K. Tsuda[9], N. Inagaki[9], Y. Seino[3], H. Koshiyama[10], KISHIMEN Investigators

[1]Cardiovascular Medicine, Kyoto University, Kyoto, [2]Kobe Gakuin University, Kobe, [3]Kansai Electric Power Hospital, Osaka, [4]Kyoto Women's University, [5]Kamo Hospital, Kyoto, [6]Kobe University, Kobe, [7]Amagasaki Hospital, Amagasaki, [8]Kyoto-Katsura Hospital, [9]Diabetes and Clinical Nutrition, Kyoto University, Kyoto, [10]Kitano Hospital, Osaka, Japan

Objective: Anti-inflammatory and lipid-lowering effects of pitavastatin have not been compared each other or between lean and overweight subjects.

Methods: Effects of pitavastatin (1-2mg, for 12 months) on high-sensitivity C-reactive protein (hs-CRP) and lipid profiles were compared among baseline body mass index (BMI) quartiles in 178 Japanese hypercholesterolemic subjects, including those with type 2 diabetes (58%), by a multi-center prospective open-label study.

Results: Baseline BMI values were significantly correlated with baseline hs-CRP (Spearman's rho=0.335, p< 0.002), but not with LDL or non-HDL cholesterol levels. Serum hs-CRP levels were significantly decreased by 4.6%, 4.4%, 16.5% and 12.8% (median values) in the 1st, 2nd, 3rd and 4th quartiles of baseline BMI, respectively. LDL cholesterol levels were significantly reduced by 27.6%, 23.6%, 32.9% and 21.9% in the 1st to 4th quartiles of baseline BMI, respectively. Non-HDL cholesterol levels were significantly lowered by 33.2%, 24.0%, 30.2% and 21.0% in the 1st to 4th quartiles of baseline BMI, respectively. Thus, pitavastatin tended to reduce hs-CRP more extensively in the higher baseline BMI quartiles; however, these differences in reduction rates of hs-CRP (p=0.934), LDL cholesterol (p=0.626) or non-HDL cholesterol (p=0.686) levels among baseline BMI quartiles were statistically insignificant. Reduction rates of hs-CRP were not significantly correlated with those of LDL cholesterol (p=0.103), non-HDL cholesterol (p=0.477) or triglycerides (p=0.368), increase rates of HDL cholesterol (p=0.264), or baseline BMI (p=0.776).

Conclusions: Pitavastatin improves lipid profiles and reduces inflammation in overweight hypercholesterolemic subjects similarly to lean subjects. Anti-inflammatory actions of pitavastatin appear to be independent of improved lipid profiles.

Memory Stick

IMPROVED LIPID REDUCTION AND TARGET ATTAINMENT WITH EZETIMIBE/SIMVASTATIN VERSUS ROSUVASTATIN IN HIGH-RISK PATIENTS FAILING PRIOR STATIN THERAPY: AGE FACTOR

Luc Missault[1], H. Vaverkova[2], M. Farnier[3], M. Averna[4], M. Viigimaa[5], Q. Dong[6], A. Shah[6], W. Taggart[6], P. Brudi[7]

[1]Department of Cardiology, St Jan Hospital, Bruge, Belgium, [2]University Hospital Olmouc, Olmouc, Czech Republic, [3]Point Medical Rond Point de la Nationmedical, Dijon, France, [4]Universita di Palermo, Palermo, Italy, [5]North-Estonia Regional Hospital, Tallinn, Estonia, [6]Merck & Co., Inc., Rahway, [7]Merck & Co., Inc., Whitehouse Station, NJ, USA

Ageing is an important factor in development and treatment of atherosclerosis. This post hoc analysis examines treatment responses in high risk patients < 65 and ≥ 65 years of age when switching from previous statin monotherapy to either ezetimibe/simvastatin 10/20 mg(E/S) or rosuvastatin 10 mg(R). Patients with hypercholesterolemia [LDL-C ≥2.6 and ≤4.9 mmol/L] entered a 6-week open-label stabilization period while continuing their prior statin. Eligible patients [LDL-C ≥2.6 and ≤4.1 mmol/L] (n=618) were randomized 1:1 to E/S or R for 6 weeks. Percent change from baseline in lipids/lipoproteins and lipid goal attainment were assessed in patients subgroups (age < 65/≥ 65). Consistency of the treatment effect across subgroups was evaluated by testing for treatment-by-subgroup interaction. No multiplicity adjustments were made. Treatment effects of E/S vs. R were generally consistent across subgroups(table). In both subgroups, E/S was more effective than R at lowering lipids and higher goal attainment rates were observed for E/S vs. R. Switching from statin monotherapy to E/S vs. R provided superior reductions in lipids and higher goal attainment in patients < 65 and ≥ 65 years of age.

Least squares mean % change from baseline (se)				LDL-C [%of patients at goal(se)] non-HDL-C			
Parameters	LDL-C	nonHDLC	TC	<2.6mmol/L	<2.0mmol/L	<3.4mmol/L	<2.6mmol/L
<65 E/S	-28 (2.1)	-23 (1.9)	-18 (1.5)	73% (3.5)	37% (3.8)	75% (3.4)	35% (3.7)
<65 R	-15 (2.3)	-12 (2.1)	- 9 (1.6)	52% (4.1)	15% (3.0)	56% (4.1)	14% (2.9)
>=65 E/S	-28(2.0)	-24 (1.8)	-17 (1.4)	72% (3.8)	39% (4.1)	74% (3.7)	39% (4.1)
>=65 R	-18 (2.1)	- 8 (2.3)	-11 (1.5)	60% (4.0)	22% (3.4)	68% (3.8)	22% (3.4)

[Table]

Memory Stick

COPPER BINDING TO S100A12: SUGGESTED ROLE IN OXIDATIVE MODIFICATION OF HUMAN LDL

Jens Pietzsch[1], M. Walther[2], R. Bergmann[1], S. Hoppmann[1]

[1]Department of Radiopharmaceutical Biology, [2]Department of Radiopharmaceutical Chemistry, Institute of Radiopharmacy, Research Center Dresden-Rossendorf, Dresden, Germany

S100A12 is a member of the S100 family of EF-hand calcium-binding proteins. S100A12 is markedly overexpressed in inflammatory compartments, and elevated serum levels of S100A12 are found in patients suffering from inflammatory and metabolic disorders, such as rheumatoid arthritis and type 2 diabetes mellitus. Besides calcium S100A12 also binds zinc and copper ions. In this regard, copper binding to S100A12 is hypothesized to influence the redox balance in (pro)inflammatory states. Both ultrafiltration assays and ^{64}Cu radionuclide binding experiments in vitro show that one molecule dimeric recombinant human S100A12 binds 2 atoms of copper (Cu^{2+}/Cu^{+}) in His motifs with a dissociation constant of about 0.02 µM. Copper-bound S100A12 can function as a pro-oxidant agent by supporting both copper reduction and copper redox-cycling, respectively. As a consequence, copper-bound S100A12 (10 µM) enhances and accelerates oxidation of human LDL lipids (decrement in *lag*-time of LDL conjugated diene formation by >15%, $p < 0.01$) and apolipoproteins (increment in apoB-100 gamma-glutamyl semialdehyde/alpha-aminoadipyl semialdehyde content by >30%, $p < 0.01$). These processes were substantially suppressed in the presence of redox-inert copper-chelating or radical-scavenging agents. It is suggested that oxidation processes mediated by copper-bound S100A12 are involved in accelerated atherogenesis in inflammatory and metabolic disorders.

Memory Stick

A DECREASE OF PLASMA TRIACYLGLYCEROL AFTER ORAL GLUCOSE LOAD PREDICTS LOWER METABOLIC RISK

Michaela Vossen, K. Toedter, U. Beisiegel, R. Müller, L. Scheja

University Hospital Hamburg-Eppendorf, Hamburg, Germany

Objectives: To relate changes of plasma lipids during an oral glucose tolerance test (OGTT) to insulin resistance and cardiovascular risk (CVR) factors.

Methods: Healthy offspring of type 2 diabetes patients (n=91) exhibiting various degrees of insulin sensitivity, as assessed by HOMA-IR, were recruited. Plasma samples collected in the fasted state and 2 hours after an oral glucose load were subjected to metabolic profiling including fatty acids (FA), lipoproteins, hormones, adipokines and inflammation markers. Data were explored statistically by correlation analysis and group comparisons.

Results: During the OGTT, concentrations of total triacylglycerol (TAG) and TAG-associated FA decreased in most subjects, whereas they rose in others. This was independent of the glucose performance. The subjects with increasing TAG (n=20) had higher BMI, WHR, HOMA-IR, and fasting glucose concentrations compared to those most efficiently lowering TAG (n=20). Furthermore, TNFa was higher and HDL-C lower in individuals with increasing TAG. Interestingly, the basal TAG and TAG-associated FA did not differ between the two groups suggesting that efficient lowering of TAG during an OGTT may be a better risk marker than fasting TAG.

Conclusions: A decrease of TAG concentrations following an oral glucose load reflects insulin action on lipid metabolism and is associated with a lower metabolic risk. In contrast, unchanged or increased TAG levels indicate a tendency for insulin resistance. Since an OGTT is performed routinely in patients with CVR, measuring of TAG as one additional parameter might gain important information.

Memory Stick

IMPACT OF SWITCHING TO SIMVASTATIN ON LOW DENSITY LIPOPROTEIN CHOLESTEROL GOAL ATTAINMENT

K Tunceli[1], S. Sajjan[2], D. Ramey[2], D. Neff[2], A. Tershakovec[2], H. Hu[1], J. Foody[3]

[1]Merck & Co., Inc., Whitehouse Station, NJ, [2]Merck & Co., Inc., North Wales, PA, [3]Harvard Medical School, Brigham and Women's, Boston, MA, USA

The goal of this retrospective, observational study was to examine the impact of switches to simvastatin on low-density-lipoprotein cholesterol (LDL-C) goal attainment among coronary heart disease (CHD) / CHD risk equivalent patients in a large US managed care claims database.

We identified 1,607,341 patients initially treated with ezetimibe/simvastatin fixed dose combination (E/S) (N=302,257), rosuvastatin (N=277,757), or atorvastatin (N=1,027,327) who were either continued on their initial therapy or switched to simvastatin between 9/1/2004 and 10/31/08. After inclusion/exclusion criteria were applied, there were 18,061 eligible patients. Mean follow-up duration was 4.3 months. Percent change from baseline LDL-C value was compared between switchers (SW) and nonswitchers (NSW) for each baseline therapy using ANCOVA. Logistic regression was used to model not achieving LDL-C goal of < 70 mg/dL (< 1.8 mmol/L) at follow-up for each baseline therapy. All analyses were adjusted for age, gender, and baseline medication potency while the logistic regressions also adjusted for baseline goal attainment.

Mean LDL-C, % at Goal and Study Outcomes Comparing SW vs NSW by Basline Therapy (N=18,061)	E/S SW (N=401)	E/S NSW (N=3,713)	rosuvastatin SW (N=93)	rosuvastatin NSW (N=2,184)	atorvastatin SW (N=427)	atorvastatin NSW (N=11,243)
Mean LDL-C mg/dL (95% CI) at baseline	76.6 (73.5, 79.8)	80.3 (79.4, 81.2)	90.3 (83.1, 97.5)	86.5 (85.3, 87.8	91.8 (89.0, 94.7)	87.1 (86.6, 87.8
Mean LDL-C mg/dL (95% CI) at follow-up	92.7 (90.0, 95.4)	81.8 (80.8, 82.8)	96.7 (91.6, 101.9)	86.7 (85.5, 88.0)	93.4 (90.9, 95.9)	87.5 (87.0, 88.0)
% at goal of <70 mg/dL (<1.8 mmol/L) (95% CI) at follow-up	19.0 (15.1, 22.8)	36.6 (35.0, 38.1)	16.1 (8.7, 23.6)	30.2 (28.3, 32.1)	17.3 (13.7, 20.9)	23.5 (22.7, 24.3)
Difference (SW-NSW) in % change in LDL-C from baseline (95% CI)	25.2 (21.2, 29.2)		13.0 (6.0, 20.0)		3.3 (0.4, 6.1)	
Odds ratio (95% CI) for not achieving LDL-C < 70 mg/dL (<1.8	3.8 (2.9, 5.1)		2.6 (1.4, 4.9)		1.6 (1.2, 2.2)	

mmol/L) at follow-up (SW vs. NSW)			

[Table]

Memory Stick

THE EFFECTS OF HYPOLIPIDEMIC REGIMEN ON LOW DENSITY LIPOPROTEIN (LDL) PHENOTYPE IN PATIENTS WITH MIXED HYPERLIPIDEMIA

Aris P Agouridis[1], T.D. Filippatos[1], E.O. Moutzouri[1], A.A. Kei[1], A.D. Tselepis[2], M.S. Elisaf[1]

[1]Department of Internal Medicine, Medical School, University of Ioannina, [2]Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece

Background: The presence of small dense LDL (sdLDL) particles in human serum is a risk factor for cardiovascular disease.

Aim: We investigated the effects of rosuvastatin, alone or in combination with fenofibrate or ω-3 fatty acids on LDL phenotype in patients with mixed hyperlipidemia.

Methods: Patients with mixed hyperlipidemia received for 3 months rosuvastatin 40 mg/day (n=25, ROS group), or rosuvastatin 10 mg/day plus fenofibrate 200 mg/day (n=20, ROSFE group) or rosuvastatin 10 mg/day plus 2gr of ω-3 fatty acids (n=25, ROSOM group). At baseline and after 3 months of treatment the serum lipid profile along with the LDL phenotype [using a 3% polyacrylamide gel-tube electrophoresis method (Lipoprint™, Quantimetrix)] were determined.

Results: In all treatment groups we observed significant reductions of plasma LDL cholesterol (LDL-C) levels: In ROS group by 61%, in ROSFE group by 40.7% and in ROSOM group by 44.3%, (all $p< 0.001$). The reduction of LDL-C was greater in the ROS group ($p< 0.001$). There was also a significant reduction of sdLDL mass (mg cholesterol/dl) in all treatment groups (all $p< 0.001$). Finally, mean LDL particle diameter was increased more in the ROSFE group compared with ROS group ($p=0.004$) and ROSOM group ($p=0.017$).

Conclusions: High doses of rosuvastatin and small doses of rosuvastatin plus fenofibrate or ω-3 fatty acids result in reductions of sdLDL-C. The combination of rosuvastatin with fenofibrate had a more favorable effect on qualitative lipoprotein abnormalities compared with rosuvastatin alone or rosuvastatin plus ω-3 fatty acids in patients with mixed hyperlipidemia.

Memory Stick

COMMON GENETIC VARIATION IN THE ATP-BINDING CASSETTE TRANSPORTER A1, LIPIDS PROFILE AND RISK OF CORONARY STENOSIS IN A TUNISIAN POPULATION

Jihène Rejeb[1], A. Omezzine[1], I. Boumaiza[1], L. Rebhi[1], K. Kchock[1], R. Belkahla[1], N. Ben Rejeb[1], N. Nabli[1], A. Ben Abdelaziz[2], E. Boughzala[3], A. Bouslama[1]

[1]Biochemistry UR MSP 28/04, [2]Information System Direction, [3]Cardiology Department, Sahloul University Hospital, Sousse, Tunisia

Objectives: ABCA1 plays an important role in HDL metabolism and atherogenesis. We studied the association of four polymorphisms (G1051A, G2706A, G2868A and -565C/T) in the ABCA1 gene with lipid profile and significant coronary stenosis (SCS) risk in a Tunisian population.

Methods: We recruited 316 Tunisian patients, who underwent coronary angiography. SCS was defined as a luminal narrowing of ≥50% in at least one major coronary artery. Genotyping was performed using PCR-RFLP.

Results: Among the four ABCA1 polymorphisms, only carriers of 2706A allele were associated with decreased risk of SCS (OR=0.458, 95% 0.22-0.92; p=0.029), without pronounced effects on HDL-C. We observed that this protective effect was also significant in smokers (OR=0.281, 95% 0.09-0.83; p=0.021). G2868A and -565C/T polymorphisms did not show any association with lipids profile or risk of SCS. Carriers of 1051A allele were significantly associated only with increased levels of HDL-C (p=0.032). When ABCA1 polymorphisms were combined in haplotypes, possessing G1051A, G2706A, G2868A and -565C/T polymorphisms, the AAGC haplotype seems to be the most protective against SCS (OR of SCS associated with haplogenotype was 0.5 (95% 1.04-3.37; p=0.048) whereas GGAT haplotype was probably the most atherogenic with an OR =1.26 (95% 1.03-1.56; p=0.025).

Conclusion: Only 2706A allele was associated with reduced risk of SCS without important modification of HDL-C levels, and it appears to be more protective for smokers than non-smokers. When the four ABCA1 polymorphisms were combined, we found that (AAGC) is a protective haplotype whereas (GGAT) haplotype have atherogenic effect against SCS in a Tunisian population.

Memory Stick

EFFECTS OF AGE AND STATIN DOSE ON LIPID LEVELS: RESULTS FROM THE VOYAGER INDIVIDUAL PATIENT DATA META-ANALYSIS

Stephen J Nicholls[1], P. Lundman[2], G. Brandrup-Wognsen[3], M. Palmer[4], P.J. Barter[5]

[1]Cleveland Clinic Foundation, Cardiovascular Medicine, Cleveland, OH, USA, [2]Danderyd Hospital, Karolinska Institute, Stockholm, [3]AstraZeneca, Mölndal, Sweden, [4]Keele University, Keele, UK, [5]Heart Research Institute, Sydney, NSW, Australia

Background: While statins have had a profound impact on cardiovascular outcomes, the impact of age on lipid responses has not been characterised.

Methods: The relationship between statin dose and changes in LDL-C, HDL-C and non-HDL-C in patients above (21%) and below 70 years was investigated in the VOYAGER individual patient (n=32258) data meta-analysis of 37 randomised, comparative studies involving rosuvastatin (R [n=18897]) and atorvastatin (A [n=15144]).

Results: A dose-dependent relationship was observed between improvements in levels of atherogenic lipids and increasing dose for both statins at all ages, with the greatest impact observed in the older patients (Table).

% change in lipids†	R 5mg	R 10mg	R 20mg	R 40mg	A 10mg	A 20mg	A 40mg	A 80mg
Age <70 years, n	578	9153	2940	2429	5974	3089	1075	1666
LDL-C	-38.4	-43.6	-49.1	-54.0	-35.2	-41.1	-45.2	-49.8
Non-HDL-C	-34.9	-39.8	-44.7	-49.3	-32.5	-37.8	-41.4	-46.2
HDL-C	5.5	6.5	7.0	8.3	4.8	3.7	2.5	2.4
Age ≥70 years, n	92	2537	614	554	1863	819	249	406
LDL-C	-41.8	-46.8*	-52.6*	-57.1*	-37.1*	-44.1*	-49.2*	-51.4
Non-HDL-C	-38.2*	-42.6*	-47.5*	-51.6*	-34.5*	-40.4*	-44.8*	-47.2
HDL-C	8.5	7.1	7.3	9.1	5.5	4.6	4.1	3.8

*p<0.05 vs patients <70 years and treated with the same statin dose; †Least square mean % change from baseline

[Table]

Conclusions: While an incremental beneficial effect on atherogenic lipids was observed with increasing dose for both statins in all patients, the greatest effect was found in older patients. This supports the need for optimal use of statins in older patients to reduce cardiovascular risk.

Memory Stick

THE HELSINKI FIELD SUBSTUDY: EFFECTS OF FENOFIBRATE AND HOMOCYSTEINE ON IN VITRO CHOLESTEROL EFFLUX POTENTIAL OF HDL AND PLASMA

Marianna Maranghi[1,2,3], A. Hiukka[2,3], R.M. Badeau[4], M. Robciuc[4], R. Vikstedt[4], R. Pahlman[4], M. Jauhiainen[4], M.-R. Taskinen[2,3]

[1]Dipartimento di Scienze Cliniche, Sapienza University of Rome, Rome, Italy, [2]Helsinki University Central Hospital, [3]Biomedicum Helsinki, [4]Public Health Genomics Research Unit, National Institute for Health and Welfare, Helsinki, Finland

Background: In the Field fenofibrate arm, HDL-C and apoA-I changes correlated inversely to changes in homocysteine (Hcy) raising the question whether Hcy counteracts fenofibrate effect on cardiovascular outcomes. Hcy-thiolactone, a metabolite of Hcy, can modify HDL apolipoproteins impairing their functions.

Aim: We investigated whether fenofibrate or Hcy modulate in vitro cholesterol efflux to HDL and plasma.

Methods: We selected 33 subjects in the fenofibrate arm according to quartiles of Hcy at 5th year: 17 subjects were in the lowest (group2) and 16 subjects were in the highest quartile (group3). In addition, 8 subjects (group1), allocated to placebo, were matched by Hcy levels to group 2. Cholesterol efflux from labeled acetyl-LDL-loaded human THP-1 foam cells to individual HDL (15 µg protein) and plasma (0.5%) from the study subjects at baseline and at 5th year were measured. The results are expressed as percentage cholesterol efflux to acceptors after normalizing data to control sample (apoA-I, 10 µg).

Results: Hcy levels at 5th year were 13.1 µmol/L (placebo), 13.3 µmol/L (fenofibrate) and 24.9 µmol/L (fenofibrate). Cholesterol efflux to HDL in the three groups was similar (group1: 0.31 - 0.42 %; group2: 0.44- 0.39%; group3 : 0.40- 0.39% at baseline vs at 5th year). In group 1 cholesterol efflux to plasma showed a tendency to increase at 5th year as compared with group 2 (0.92% versus 0.73%in group2 at 5th year).

Conclusions: Fenofibrate treatment and high Hcy levels have no effect on either HDL or plasma potential to remove cholesterol from foam cells.

Memory Stick

EFFECTS OF EZETIMIBE ADDED TO ON-GOING LOW-DOSE PRAVASTATIN ON SERUM LIPOPROTEIN, CHOLESTEROL SYNTHESIS AND ABSORPTION IN HYPERCHOLESTEROLEMIC JAPANESE PATIENTS

Jun Sasaki[1], S. Biro[2], T. Saikawa[3], S. Kono[4]

[1]Graduate School of Pharmaceutical Medicine, International University of Heath and Welfare, Fukuoka, [2]Tsukasa Hospital, Kagoshima, [3]Ooita University, Ooita, [4]Kyushu University, Fukuoka, Japan

Objective: A randomized trial was implemented to evaluate effects of ezetimibe (EZE) add-on to pravastatin (PRA) on the levels of serum lipoprotein, cholesterol synthesis and absorption in Japanese.

Methods: Study subjects were those who had LDL cholesterol of 120 mg/dL or greater with low-dose PRA(5,10mg/day) treatment, and were randomly allocated to either EZE (10mg/day) add-on or double-dose PRA treatment, and follow-up measurements were done after 3 months. The cholesterol synthesis levels were measured the serum level of lathosterol (LAT), a biomarker of synthesis, and absorption levels were measured by serum sitosterol (SIT) and campesterol (CAM).

Results: 96 and 95 patients were allocated to EZE and PRA, respectively. LDL cholesterol and apo B decreased by 15.6% and 13.9% in the EZE group, and by 5.9% and 4.4% in the PRA group, respectively. Triglycerides decreased by 4.8% in the EZE group and increased by 17.4% in the PRA group. Differences in the decreases were all statistically significant. LAT increased by 76.0% in the EZE group and 24.2% in the PRA group, respectively. SIT and CAM decreased by 48.0% and 36.3% in the EZE group, respectively, and increased by 17.0% and 14.1% in the PRA group, respectively. The differences between both groups were all statistically significant. Both treatments were well tolerated.

Conclusions: These findings suggest that EZE added on low-dose PRA is more efficient for lipid management compared with the doubling dose of PRA. EZE inhibits cholesterol absorption and increases cholesterol synthesis.

Memory Stick

COMPARATIVE EFFECT OF APHERESIS VS ATORVASTATIN/APHERESIS ON MARKERS OF INFLAMMATION IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Mariarita Puntoni[1], F. Bigazzi[2], F. Sbrana[2], B. Pennato[3], F. Minichilli[1], T. Sampietro[2]

[1]CNR Institute of Clinical Physiology, [2]Gabriele Monasterio CNR Regione Toscana Foundation of Pisa, [3]Scuola Superiore Sant'Anna, Pisa, Italy

Objective: Patients with familial hypercholesterolemia (FH) have increased cardiovascular events. Lowering circulating lipid by LDL-apheresis has beneficial effects on prognosis. Apheresis also improves endothelial dysfunction in FH as assed by skin laser Doppler blood flow. Whether apheresis vascular effects in FH are related to modulation of pro- and anti-inflammatory cytokines, and whether the combination of apheresis with atorvastatin enhance the putative anti-inflammatory effect remains unknown. We examined the effect of atorvastatin/apheresis vs. apheresis alone on the releasing of circulating pro- and anti-inflammatory markers.

Methods: 9 heterozygous FH patients (56±11 years, mean CH 385±42 mg/dL) were treated with apheresis and with apheresis plus atorvastatin 40 mg/d. Lipid profiles, TNFa, CRP, CK, GOT, GlT, anti-inflammatory markers IL-4 and IL-10 and pro-inflammatory markers INFg, IL-2, and IL-6 were determined before and at 2, 4, 6 and 8 days after apheresis and atorvastatin/apheresis.

Results: Treatment with atorvastatin/apheresis significantly improves lipid profile more than LDL-apheresis alone at each scheduled time. When compared to apheresis alone, combined treatment decreased CH by more than 25-35% at all times and increased IL-4 concentration. The cholesterol levels in atorvastatin/apheresis patients were inversely correlated with IL-4 and IL-10 and positively correlated with IFNg.

Conclusion: The combination of atorvastatin/LDL-apheresis decreased serum CH more than apheresis alone. Apheresis had an anti-inflammatory effect. However, the addition of atorvastatin increased the concentration of anti-inflammatory cytokine IL-4, suggesting that the drug reducing cholesterol levels affects the balance between pro- and anti-inflammatory cytokines towards the anti-inflammation contribution and to the restoration of vascular function.

Memory Stick

HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (FH) IN GREECE

Euridiki Drogari[1], P. Progias[1], A. Skouma[1], M. Elisaf[2], V. Mollaki[1]

[1]1st Department of Pediatrics, Choremio Research Laboratory, Unit of Metabolic Diseases, Aghia Sophia Children´s Hospital, University of Athens, Medical School, Athens, [2]Department of Internal Medicine, Medical School, University of Ioannina, Ioannina, Greece

FH is an autosomal dominant disease with a frequency of 1:500 and $1:10^6$ in its heterozygous and homozygous form, respectively. It is mainly mutations in the Low Density Lipoprotein Receptor gene (*LDLR*) that cause high LDL and total cholesterol levels. Homozygous patients show a 6 to 8-fold increase in LDL levels, premature atherosclerosis and myocardial infarction before the 3rd decade of life. Therefore, it is critical to identify and diagnose the disease in early childhood. The aim of the study was the clinical and molecular diagnosis of homozygous FH children and adults in Greece. Forty clinically diagnosed homozygous FH patients from 37 families were examined. The molecular diagnosis was performed with direct DNA sequencing of the *LDLR* gene or restriction fragment length polymorphism analysis. The patients showed 480-2000 mg/dL total cholesterol, 4 of them had corneal arcus and all had tendom xanthomas. We found 12 Gly571Glu homozygotes, 6 Val408Met homozygotes, 5 Gly528Asp homozygotes, 3 Ser265Arg homozygotes, and 1 Cys6Trp homozygote. We also found 4 compound heterozygotes Ser265Arg/Val408Met and 6 compound heterozygotes Gly528Asp/Ser265Arg, Gly528Asp/Gln233Pro, Ser265Arg/Cys152Arg, Cys6Trp/Ile420Asn, Ser265Arg/Cys313Stop, and Val408Met/Cys313Stop, respectively. The number of homozygous patients confirms that the disease is underdiagnosed, at least in Greece, since consaguinity is not common. The lipid levels were different among the homozygotes carrying the same mutation(s), suggesting that there are other genetic and/or environmental factors affecting cholesterol metabolsim.

Memory Stick

LOW HDL-HIGH INFLAMMATORY MARKERS IN HEART FAILURE INDUCED BY HIGH FREQUENCY PACING IN MINIPIGS

Benedetto Pennato[1], V. Lionetti[1], F. Bigazzi[2], M. Puntoni[3], A. Simioniuc[1], M. Campan[1], F. Recchia[1], T. Sampietro[2]

[1]Scuola Superiore Sant'Anna, [2]Gabriele Monasterio CNR Regione Toscana Foundation of Pisa, [3]CNR Institute of Clinical Physiology, Pisa, Italy

Background: Many studies suggest a major role for high-density lipoproteins (HDL) in the vascular homeostasis, not necessarily related to pro- or anti-atherosclerotic mechanisms. Low HDL, together with a pro-inflammatory state, seem to be associated with left ventricular dysfunction in the absence of coronary atherosclerotic lesions, as occurs in idiopathic dilated cardiomyopathy.

Aim: To test possible correlations between the development of non-ischemic cardiac failure and altered levels of HDL and ApoAI, inflammatory markers C3, a2-macroglubulin and ceruplasmin, in a pig model of pacing-induced dilated cardiomyopathy.

Material and methods: 8 adult male minipigs were chronically instrumented with a pacemaker connected to the left ventricular (LV) wall. Blood samples were collected at baseline, i.e. before starting the pacing protocol, and after three weeks of pacing at 180 beats/min, when LV ejection fraction was < 35% and end-diastolic pressure was >18 mmHg.

Results: After three weeks of pacing there were no significant changes in total cholesterol and triglycerides compared to baseline (57±7.50 vs 53.88±13.79 mg/dl and 23.67±9.31 vs 28.43±4.50 mg/dl respectively). Conversely, HDL and ApoAI levels were dramatically decreased (21.63±2.45 vs 9.63±3.62 mg/dl, $p< 0.0005$, and 16.86±0.97 vs 9.76±3.41 mg/dl, $p< 0.005$). Among the inflammatory markers, a2-macroglubulin and ceruplasmin levels were significantly increased (107.14±15.65 vs 134.35±26.04 mg/dl, $p< 0.05$, and 26.51±3.37 vs 36.45±5.92, $p< 0.01$), while C3 levels were not significantly changed (14.66±4.59 vs 17.88±8.30 mg/dl).

Conclusion: Our results suggest a novel association between development of cardiac dysfunction and HDL decrease, even in the absence of co-morbidities and alterations of total cholesterol and triglycerides.

Memory Stick

SERUM APOLIPOPROTEIN B-48 LEVEL IS CORRELATED WITH CAROTID INTIMA-MEDIA THICKNESS (IMT) IN SUBJECTS WITH NORMAL SERUM TRIGLYCERIDE LEVEL

Kazuhiro Nakatani[1], T. Sugimoto[2], R. Kawase[1], H. Nakaoka[1], M. Inagaki[1], M. Yuasa-Kawase[1], K. Tsubakio-Yamamoto[1], J. C Sandoval[1], D. Masuda[1], T. Ohama[1], Y. Nakagawa-Toyama[1,3], M. Nishida[1,3], M. Ishigami[1,4], I. Komuro[1], S. Yamashita[1], Cardiolipidology

[1]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, [2]Internal Medicine, Sugimoto Clinic, Osaka, [3]Health Care Center, Osaka University, Toyonaka, [4]Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, Suita, Japan

Background: Severe atherosclerotic cardiovascular diseases are frequently observed among subjects with normal TG values. It has not been clarified yet whether metabolic profile of these subjects is proatherogenic or not.

Aim: To investigate the correlation between lipoprotein profiles and intima media thickness (IMT) of carotid arteries.

Methods and results: Clinical profiles and IMT were measured in subjects (n=245) who came for an annual health checkup. They were divided into 3 groups by fasting TG levels (group N-1; TG < 100 mg/dl, N-2; 100 ≦ TG < 150 mg/dl and H; 150 mg/dl≦TG). Waist circumference, BMI, fasting levels of apoB-48, apoB-100, remnant lipoprotein cholesterol (RemL-C), uric acid, and TG concentrations in the CM, VLDL and LDL fractions of group N-2 were significantly higher than those of group N-1. There was no significant difference in mean IMT between the two groups. When subjects in each group were divided into two groups by IMT levels (High IMT group: IMT≧ 1.1 mm and Low IMT group: IMT < 1.1 mm), fasting apoB-48 was significantly higher in High IMT group than in Low IMT group. However, there was no significant difference in the fasting levels of apoB-100 and RemL-C between the two groups.

Conclusion: These data suggest that the accumulation of exogenous lipoproteins might be one of the risk factors for the development of atherosclerosis among subjects with normal TG levels and that the measurement of fasting apoB-48 levels is useful for the risk assessment of atherosclerotic cardiovascular diseases.

Memory Stick

TRANSFER OF LIPIDS TO HDL IN PATIENTS WITH PRECOCIOUS ISCHEMIC HEART DISEASE

Raul Maranhão[1,2], F.R.S. Freitas[1], A.P. Mansur[1], C. Strunz[1], A.T. Yamada[1], A.J. Mansur[1]

[1]Heart Institute, Medical School Hospital, University of Sao Paulo, São Paulo, [2]Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil

Background: It has been increasingly perceived that systematically assessing metabolic and functional aspects of HDL are important for the understanding of HDL protective role against atherogenesis. In this metabolism, lipid exchanges between HDL and the other lipoprotein classes is determinant of HDL composition and function in cholesterol esterification and in reverse cholesterol transfer.

Objective: Test the hypothesis whether the *in vitro* simultaneous transfer to HDL of free-cholesterol, cholesteryl esters, phospholipids and triglycerides is different in patients with precocious ischemic heart disease (IHD) and age-sex paired controls without disease.

Methods: Fifty-five patients with IHD and 54 healthy subjects were studied (45-55y). Fasting plasma samples were incubated for 1h with an artificially-made nanoemulsion labeled with radioactive lipids and prepared by ultrasonication; radioactive lipids transferred from the donor nanoemulsion to HDL were quantified in the supernatant after chemical precipitation of non-HDL fractions and the nanoemulsion.

Results: Serum total, LDL cholesterol and triglycerides were higher in IHD patients than in controls, but HDL cholesterol was equal. The transfers to HDL of free cholesterol (7.6±3.3; 3.9±1.1), phospholipids (19.4±3.5; 17.9±3.3) and triglycerides (5.0±1.7; 3.7±1.5) were lower in IHD patients than in controls, whereas the cholesteryl esters transfer was higher in IHD patients (4.5±1.6; 6.7±2.5) than in non-IHD.

Conclusion: The marked alterations in lipid transfers to HDL precocious in IHD patients probably results in dynamic alterations in the composition and stability of the lipoprotein and thereby may alter HDL-related antiatherogenic functions and may serve as disease markers or therapeutic targets.

Memory Stick

APOLIPOPROTEINS IN THE DISCRIMINATION OF ATHEROSCLEROTIC BURDEN AND CARDIAC FUNCTION IN PATIENTS WITH CORONARY ARTERY DISEASE

A. Abraheem[1], P. Jeetesh[2], M. Gunning[3], J. Creamer[3], E. Hughs[2], G. Lip[2], **Mohamed Elhassan**[1]

[1]Tameside General Hospital, Manchester, [2]City Hospital, Birmingham, [3]UHNS, Stoke-on-Trent, UK

Purpose: To compare the performance of apolipoproteins and oxidised LDL against routine clinical lipid profiles in the discrimination of atherosclerotic burden and cardiac function.

Methods: Using a cross-sectional approach, we measured oxidised LDL, Apo AI and B in 200 patients (34-81 years) and routine biochemical parameters with symptomatic, but stable CAD, undergoing diagnostic coronary angiography.

Main outcome measures: The discrimination of

(i) atherosclerotic burden: coronary atheroma scores, the number of diseased coronary vessels, and

(ii) cardiac function: NYHA classification, left ventricular systolic dysfunction (LVSD) was judged using Receiver Operating Characteristic (ROC) curves.

Results: The ratio of Apo AI to B was closely correlated to oxidised LDL (Spearman, r = 0.37, p< 0.001). However, levels of oxidised LDL were unrelated to measures of cardiac function or coronary disease severity. Concentrations of Apo AI decreased with increasing atheroma scores (P=0.02), while triglyceride levels increased (P = 0.016), and these associations remained significant after adjusting for age, gender and heart failure status. HDL cholesterol and Apo AI was higher amongst those with heart failure (P=0.002), and levels increased in an ordinal fashion with NYHA class (P = 0.005). On ROC analysis, reduced levels of Apo AI and HDL cholesterol were consistent discriminators for patients in the upper quartile for atheroma score (P < = 0.004).

Conclusion: ApoA1 and B levels reflect both qualitative and quantitative aspects of lipid metabolism, and the Apo A1 is a consistent discriminator of atherosclerotic burden amongst patients with stable CAD.

Memory Stick

IN VITRO AND *IN VIVO* POLYPHENOL BINDING TO LIPOPROTEINS: A PROTECTIVE MECHANISM FOR ATHEROSCLEROSIS

Joe Vinson

Chemistry, University of Scranton, Scranton, PA, USA

Introduction: The mechanism for the beneficial health effects of polyphenols is under intensive investigation and it goes beyond a simple antioxidant effect to include gene expression, cell signaling, enzyme activation and inhibition. The oxidative theory of atherosclerosis hypothesizes that antioxidants should be beneficial. An antioxidant mechanism might be important for polyphenols and atherosclerosis as there is a large epidemiological and experimental basis for polyphenols' CHD benefit.

Objective: To determine if polyphenols bind to lipoproteins and are protective for oxidation.

Results: Our results demonstrate atherogenic lipoproteins LDL+VLDL bind *in vitro* to polyphenols from 8 representative classes, several metabolites, and from a green tea extract. The binding (calculated K_d from 10^3-$10^5 M^{-1}$) occurs at both inflammation pH 5.2 and physiological pH 7.4. Competition experiments with physiological ratios of the major plasma protein albumin and LDL+VLDL indicate that polyphenols bind with reduced capacity. Bound polyphenols and metabolites, concentrated in the lipoprotein particles, act as significant antioxidants at nM levels. In a human study demonstrating *in vivo* binding, one subject was sampled 2 hours after drinking in consecutive weeks 240 ml of coffee, green tea, black tea, dealcoholized red wine or prune juice. Isolated LDL+VLDL was protected from *ex vivo* oxidation.

Conclusion: Polyphenols bind to lipoproteins and are antioxidants even after metabolism which is a protective mechanism for atherosclerosis.

Memory Stick

EFFECTS ON CVD RISK FACTORS OF ADDING GROUND, SLICED AND WHOLE HAZELNUTS TO CUSTOMARY DIETS OF HYPERCHOLESTEROLAEMIC MEN AND WOMEN

Alexandra Chisholm[1], S. Tey[1], R. Brown[1], A. Gray[2], C. Delahunty[3]

[1]Human Nutrition, [2]Preventive and Social Medicine, University of Otago, Dunedin, New Zealand, [3]Food Science Australia, CSIRO, North Ryde, NSW, Australia

Background: Diets regularly including nuts are associated with beneficial changes in lipid and lipoprotein concentrations and reductions in risk of cardiovascular disease (CVD). Long term acceptability of frequent consumption and possible effects of different physical forms of hazelnuts are unclear.

Objective: To assess the effects on CVD risk factors of incorporating ground, sliced and whole hazelnuts into the usual diet, and to investigate acceptability of 30g/day consumed over three months.

Methods: Forty eight mildly hypercholesterolaemic individuals were recruited from the general public, mean (SD) age 49.9(9.4) years and BMI 25.9(3.5)kg/m^2. Baseline mean (SD) lipids TC 5.86(0.67)mmol/l, LDL-C 4.00 (0.67)mmol/l, HDL-C 1.22(0.34)mmol/l, TAG 1.41(0.64)mmol/l. Intervention was a randomised, multiple crossover with three four week dietary phases followed by two-week washouts. Diet was assessed with 3-day weighed records at baseline and during each intervention. Acceptance of hazelnuts rated daily on visual analogue scales. Statistical analyses used multiple regression and paired t-tests (Stata Intercooled version 9.0).

Results: Consuming 30g hazelnuts for 3 months increased plasma HDL ($p= 0.023$) and α-tocopherol ($p=0.005$), reduced TC, LDL-C, TC:HDL ratio (all $p< 0.001$); apoB100 ($p=0.002$) and apoB100:apoA1 ratio ($p< 0.001$) without changing body weight ($p=0.813$) or triglycerides ($p= 0.725$). Dietary total fat, monounsaturated fat and α-tocopherol increased and carbohydrate decreased, (all $p< 0.001$). Saturated and polyunsaturated fat, protein and dietary cholesterol were unchanged (all $p>0.357$). Acceptance for nuts was unchanged during the intervention.

Conclusion: These data suggest that the addition of hazelnuts to the usual diet has potential to reduce CVD risk in mildly hyperlipidaemic persons.

Memory Stick

AN 8-WEEK TRIAL INVESTIGATING THE EFFICACY AND TOLERABILITY OF ATORVASTATIN IN CHILDREN AND ADOLESCENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HEFH)

Kuan Gandelman[1], P. Glue[2], R. Laskey[1], J. Jones[1], R. Labadie[1], L. Ose[3]

[1]Pfizer Inc., New York, NY, USA, [2]University of Otago, Dunedin, New Zealand, [3]Rikshospitalet, Oslo, Norway

Objective: To evaluate the efficacy and tolerability of atorvastatin in Tanner Stage (TS) 1 (aged 6-10 years) and TS≥2 (aged 10-17 years) patients with HeFH.

Methods: In an open-label, 8-week study, 15 TS1 and 24 TS≥2 children were treated initially with 5 mg atorvastatin chewable tablets or 10 mg atorvastatin marketed tablets, respectively; doses were doubled at Week 4 if the lipid target was not achieved. Efficacy variables were percent change-from-baseline in LDL-C, total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), apolipoprotein (Apo) A-I, and Apo B. Safety evaluations included clinical monitoring, subject-reported adverse events (AEs), vital signs, and clinical laboratory tests.

Results: Mean values for LDL-C, TC, VLDL-C and Apo B decreased by Week 2 among all TS1 and TS≥2 subjects, whereas triglycerides, HDL-C and Apo A-I varied from week to week. After 8 weeks, mean (SD) reduction in LDL-C in TS1 children was −40.7% (8.4) and in TS≥2 children was −39.7% (10.3). In TS1 subjects, mean reductions in TC and triglycerides were −34.1% (6.9) and −6.0% (32.1), respectively. Corresponding changes in TS≥2 subjects for TC and triglycerides were −35.6% (9.5) and −21.1% (29.7). Four subjects experienced mild to moderate treatment-related AEs; no serious AEs or discontinuations were reported. Overall, no difference in safety and tolerability was observed between TS1 and TS≥2 cohorts.

Conclusions: Following 5-10 mg (TS1) or 10-20 mg (TS≥2) doses, clinically meaningful reductions in lipid parameters were observed with atorvastatin in children with HeFH. Atorvastatin was well tolerated in this population.

Memory Stick

DEFICIENT ANTIOXIDATIVE FUNCTION OF HDL PARTICLES IN LOW HDL-CHOLESTEROL DYSLIPIDEMIAS: IMPACT OF THE DEGREE OF TRIGLYCERIDEMIA

H. Hussein[1,2,3], L. Tokgözoğlu[4], S. Chantepie[1,2,3], P. Giral[1,3], B. Hansel[1,3], M. Alikasifoglu[4], M. Ercan[4], M.J. Chapman[1,2,3], **Anatol Kontush**[1,2,3]

[1]INSERM Unit 939, Hopital de la Pitie, [2]Université Pierre et Marie Curie-Paris 6, [3]AP-HP, Groupe Hospitalier Pitié-Salpétrière, Paris, France, [4]Department of Cardiology, Hacettepe University, Ankara, Turkey

Small, dense HDL3 potently protect LDL from oxidative stress; such activity is diminished in metabolic diseases involving hypertriglyceridemia and low high-density lipoprotein-cholesterol (HDL-C) levels. Molecular determinants of such defective antioxidative function however remain indeterminate.

Two distinct low HDL-C populations (non-obese subjects of Turkish origin and subjects with Metabolic Syndrome, MetS) displaying distinct degrees of triglyceridemia were studied. The capacity of both small HDL3c and total HDL to protect LDL from oxidation was progressively impaired in parallel with the degree of triglyceridemia (up to -79%, $p< 0.05$) in both low HDL-C populations as compared to normolipidemic controls. Apolipoprotein A-I (up to -21%, $p< 0.01$), monounsaturated fatty acids (MUFA; up to -23%, $p< 0.001$) and cholesteryl esters (CE; up to -64%, $p< 0.001$) were deficient in HDL of both dyslipidemic populations, which were enriched in saturated fatty acids (SFA; up to +20%, $p< 0.05$) and TG (up to +189%, $p< 0.01$), resulting in elevated SFA/MUFA and TG/CE ratios. The rigidity of surface lipids was elevated in HDL3 from both low HDL-C populations ($p< 0.05$).

We conlude that antioxidative activity of HDL3 is progressively impaired in low HDL-C subjects in parallel with the degree of triglyceridemia reflecting diminished apoA-I content, enhanced surface lipid rigidity and elevated SFA/MUFA and TG/CE ratios.

Memory Stick

WHEN IS THE DYSLIPIDEMIA BEGINNING IN FACT? THE IMPAIRED LIPID METABOLISM (ILM)

Istvan Reiber[1], I. Mező[2]

[1]4th Dept Internal Med., [2]2nd Dept Internal Med., Szent Gyorgy Hospital, Székesfehérvár, Hungary

Background and aims: Elevated fasting triglycerides (TG) are controversal risk factors for cardiovascular disease. At the time we measured in fasting state the typical atherogenic lipid parameters, our patients have already the lipid disorder since years or decads. The aim of the study was to investigate how can we earlier and more correctly define the impaired lipid status of our patients.

Patients and methods: Fasting (FA) and postprandial (3 hours after usual breakfast (PAB) and after usual lunch (PAL)) serum of 156 non diabetic, lipid lowering drugs naive subjects were collected and analyzed for lipids (total-cholesterol (TC), HDL-C, TG, apoB, apoCIII, apoE) and glucose.

Results: We have 3 groups of patients: I. FA-TG: < 1.0 mmol/L (n=52), II. FA-TG: 1.0-1.7 mmol/L (n=52) and III. FA-TG: > 1.7 mmol/L (n=52). All the pts have FA-TC < 5.20 mmol/L (mean: 4.51±0.62 mmol/L) and FA-HDL-C: 1.40±0.42 mmol/L (ns differences between the groups). There were no significant change in the postprandial TC. We detected higher levels of fasting apoCIII and apoB in the III. group. Not surprisingly, there were significant ($p < 0.05$) differences between FA-TG and PAB-TG and PAL-TG. For all subjects in group I. nonfasting TG were < 2.0 mmol/L.

Conclusions: We can detect already ILM, when pts have normal fasting lipid quantity or qualitatively abnormal lipids. ILM can be diagnosed earlier if we measure the nonfasting lipid levels. Anytime during the day, if TG > 2.0 mmol/L together with elevated apoB (or atherogen cholesterol=non-HDL-C) and decreased HDL-C, it is time for intervention.

Memory Stick

PRELIMINARY BIOCHEMICAL AND MOLECULAR RESULTS OF PORTUGUESE FAMILIAL COMBINED HYPERLIPIDAEMIA PATIENTS

Tânia Santos[1], Q. Rato[2], A. Guerra[3], I.M. Gaspar[4], M. Bourbon[1]

[1]Dpt de Promoção da Saúde e Doenças Crónicas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, [2]Dpt. de Cardiologia, Hospital S. Bernardo, Setúbal, [3]Dpt. de Pediatria, Hospital S. João, Porto, [4]Dpt. de Genética Médica, Hospital Egas Moniz, Lisboa, Portugal

The aim of this study is the biochemical and molecular characterization of Familial Combined Hyperlipidaemia (FCHL) patients.

Twenty seven patients with FCHL phenotype were studied. Molecular study of *LPL*, *APOAIV*, *APOAV*, *APOCII*, *APOCIII* and *USF1* (s1, s2) regions was performed by PCR amplification and sequencing. Serum ApoA-IV and ApoA-V were measured by ELISA and LDL subfractions were analysed by lipid electrophoresis (Lipoprint®).

Twenty one patients showed alterations in the genes analysed. *LPL* A433V, APOAIV Q359_E362del and *APOAV* D332fsX336 were not described before. *LPL* A433V was found in 2% of 50 normolipidaemic controls. The Q359_E362del patient had ApoA-IV concentration (15.5 mg/dL) within normal range. The patient carrying the D332fsX336 showed low ApoA-V concentration (74.5 ng/ml). FCHL patients showed predominance of sdLDL even under medication with statins, unlike Familial Hypercholesterolaemia (FH) patients, which have larger LDL when taking statins.

From the results obtained, *LPL* A433V seems to be a polymorphism. APOAIV Q359_E362del needs further investigation to determine protein function and *APOAV* D332fsX336 studies seem to indicate that this alteration is a mutation causing disease. Lipoprint® analysis was found to be a useful marker to distinguish FCHL and FH patients but larger number of patients will be studied to confirm these results. FCHL has a very complex phenotype, characterized by highly atherogenic lipoprotein profile and increased cardiovascular risk. Biochemical characterization and genetic identification of alterations that influence FCHL phenotype are important to determine CVD risk allowing for a better choice of therapeutic measures.

Memory Stick

PARADOXICAL DECREASE IN HIGH DENSITY LIPOPROTEIN CHOLESTEROL WITH FENOFIBRATE: A QUITE RARE PHENOMENON INDEED

Giuliana Mombelli[1], F. Pazzucconi[1], A. Bondioli[1], A. Zanaboni[2], S. Gaito[2], L. Calabresi[3], C.R. Sirtori[1,3]

[1]University Center for Dyslipidemias, Niguarda Hospital, [2]Department of Computer Sciences, [3]Department of Pharmacological Sciences, University of Milan, Milan, Italy

Some recent clinical reports have suggested that paradoxical decreases in high density lipoprotein cholesterol (HDL-C) levels after fenofibrate treatment may be quite common. These appear to occur mainly in patients with combined fibrate/statin therapy and possibly in those with low baseline HDL-C. Reports on HDL-C reductions after fenofibrate are possibly supported by the disappointing results in terms of HDL-C responses from the recent FIELD study.

A survey on 581 patients treated for one year or longer was carried out in our Clinical Center. This indicated that paradoxical HDL-C reductions are a relatively uncommon phenomenon. Not more than 15.3% of the present series showed an HDL-C reduction, mostly of a modest degree. Further, reductions of HDL-C appear to occur mainly in individuals with significant HDL-C elevations (> 50 mg/dl), almost never in patients with low HDL-C. Otherwise, there seems to be no impact of a previous diagnosis of diabetes or hypertension on the HDL-C changes. From a very recent pharmacogenomic study on the apo A1/C3/A4/A5 gene cluster, genetic influences appear only to reduce the positive impact of fenofibrate on HDL-C, but do not indicate any risk of occurrence of HDL-C reductions.

Also based on our very long experience with this drug, it appears that fenofibrate raises HDL-C levels in the vast majority of treated patient , with a particularly dramatic effect in individuals with low HDL-C and hypertriglyceridemia.

Memory Stick

CAPILLARY ISOTACHOPHORESIS AS AN OVERALL TOOL TO REVEAL ATHEROGENIC LIPOPROTEIN PHENOTYPE

Alexander Dergunov[1], S. Visvikis-Siest[2], G. Siest[2]

[1]Biochemistry, National Research Centre for Preventive Medicine, Moscow, Russia, [2]INSERM U525 - Equipe 4, EA 4003, Université Henri Poincaré - Faculte de Pharmacie, Nancy, France

Object: Hypertriglyceridemia, diminished HDL content and the presence of small dense LDL are common features of atherogenic lipoprotein phenotype. Besides, the accumulation of nascent HDL with pre-beta electrophorertic mobility is suggested to reflect the deficiency of reverse cholesterol transport.

Results and conclusion: The original approach was developed to follow 11-12 individual lipoproteins by capillary isotachophoresis (CITP) of parallel plasma aliquots pre-stained with lipid-specific fluorescent probe or fluorescein-labeled apoE. The slow HDL with pre-beta mobility (sHDL) and fast LDL with the increased negative charge (fLDL) were precisely localized in lipid and apolipoprotein CITP profiles. For 10 patients with ε3/ε3 genotype and plasma triglyceride content varying in a wide range, the fLDL content was positively associated with VLDL while negatively with HDL cholesterol, thus evidencing the fLDL accumulation in hypertriglyceridemia. Besides, apoE content in sHDL increased at the decrease of HDL cholesterol. The affinity parameters of apoE binding to individual lipoproteins were measured at plasma titration by apoC-III, followed by residual apoE detection. These parameters for sHDL and fLDL were associated positively, assuming the common metabolic pathway(s). This metabolomic-like approach is suggested to permit the overall, fast and quantitative determination of atherogenic lipoprotein phenotype.

Memory Stick

WOMEN´ S HEALTH: ESTROGEN, PROGESTINS AND BLOOD PRESSURE

Ruth-Maria Korth

Research in General Medicine Fida, Lehrauftrag LMU, Munich, Germany

Aim: A hypertensive risk was invented here with women during pregnancy, intolerance to glucose (IGTT:www.fidabus.com) or with hormone users or with non-pregnant women at metabolic risk beyond hormone use.

Methods: Initial biomarkers were anonymously enrolled and oral glucose tolerance was tested (1h-50g-oGTT Reflotron Roche, R.M.Korth, jmhg 2006).

Results: First, pregnant women with reversible IGTT had values between 140-169 mg/dl glucose (16% of 180, aged 31±5 years, 23±4 kg/m2) reporting healthy food and lifestyle. Normal blood pressure was observed (week 22±2: 104±13/64±11 mmHg; week 38±2: 110±8/73±5 mmHg, ±1S.D.). Pregnant women with persisting control-IGTT tended to gestation diabetes elsewhere (4% ≥170 mg/dl, 1-h-100g glucose). Second, oral contraceptives were not correlated with raised blood pressure ($p>0.1$) probably as weight and lipids were normal (n=49, 22±4 kg/m2, aged 27±7 years, 117±13/82±9 mmHg, LDL: 127±36, HDL: 66±14 mg/dl). Third, aging women with menopausal HRT-hormones (15 out of 46, aged 51±9 years, 25±5 kg/m2) were compared to those without HRT and led away from raised blood pressure ($p>0.35$). Overall, non-pregnant women with IGTT and mixed hyperlipidemia showed hypertension (n=14, aged 32±10 years, 26±6 kg/m2, LDL: 167±65, HDL: 54±9,Trig:254±73 mg/ml, 136±25/97±12 mmHg). Indeed, women with IGTT or mixed hyperlipidemia had significantly higher blood pressure than appropriate controls ($p< 0.003$) and multivariate analysis provided evidence for direct risk of diastolic hypertension ($p< 0.05$). This model is suitable to adress hormonal compositions without hypertension as only participants with IGTT-hyperinsulinemia or mixed hyperlipidemia were at direct risk for diastolic hypertension.

Memory Stick

MODIFIED, EXTRACTED BARLEY BETA GLUCAN (BBG) EFFECTIVELY LOWERS LDL CHOLESTEROL DESPITE REDUCED VISCOSITY

Joseph Keenan

Dept of Family Medicine, University of Minnesota, Minneapolis, MN, USA

Many studies have shown that foods rich in viscous fibers can lower LDL- C. This study compared the effect on blood lipids of a food and beverage fortified with unmodified BBG (HMW), average molecular weight of 1000 kDa, to the effect of the same food and beverage fortified with modified BBG (LMW), average molecular weight 50-400 kDa in persons with moderate dyslipidemia. Subjects(N=155) with LDL-C levels between 130 and 190 mg/dl after a 4 week run in period on a “heart-healthy” diet were randomized to 5 parallel treatment arms (5g/d HMW, 5g/d LMW, 3g/d HMW, 3g/d LMW or control). Over the six week treatment period subjects consumed one serving per day of a fortified cereal and one serving per day of a fortified beverage or control.

Results: Change from baseline in LDL-C (5g HMW = - 22.5 mg/dl (-14.6%); 5g LMW = - 20.3mg/dl (-13.1%); 3g HMW = -14mg/dl (-9%); 3g LMW = - 13.4mg/dl (-9%). There was good compliance with all treatments with all subject groups consuming 94+% of servings and there were no drop outs or significant differences in side effects reported except for increased intestinal gas in the 5g HMW compared to control.

Memory Stick

INTERRELATION BETWEEN OBSTRUCTIVE SLEEP APNEA AND LIPID SPECTRUM AMONG CARDIOVASCULAR PATIENTS

Natalya Belinskaya[1], L. Yashina[2]

[1]Sleep Medicine Center, [2]Polyclinic Therapy Department, Chelyabinsk State Medical University, Chelyabinsk, Russia

Introduction: It has been proved in the research that people suffering from obstructive sleep apnea (OSA) are subject to high risk of atherosclerosis, hypertensive disease and death.

Objectives: Research prevalence of OSA among cardiovascular patients. Research interrelation between obstructive sleep apnea and lipid spectrum among cardiovascular patients.

Material and methods: 242 patients with hypertensive disease and coronary heart disease with clinical signs of OSA have been examined. There were 172 men and 70 women, their age was from 32 to 65 years. Polysomnography, lipidogram, glucose and uric acid have been investigated.

Results of research: Significant forms of OSA (apnea-hypopnea index is more than 15 per hour) have been reveled among 67 patients (27.6%), there were truly more men (41.3%) than women - - 8.6% ($p < 0.05$). Hypercholesterolemia (LDL is more than 2.5 mmole/l) was discovered among 52% of the patients suffering from OSA against 18% without OSA. Reduced level of HDL-C was more often found among the patients with OSA - 69% against 38% ($p < 0.05$). Fasting hyperglycemia and impaired glucose tolerance was found among 12% with OSA and 11% without OSA; hyperuricemia - among 42% of the patients with OSA and 8% without OSA ($p < 0.05$).

Conclusions: Thus, men with cardiovascular diseases are suffering definitely more often from OSA. Patients with SOAS more often have hyperuricemia, hypercholesterolemia and reduced level of HDL-C.

Memory Stick

ATHEROGENIC DISLIPIDEMIA IN METABOLIC SYNDROME: ROLE OF INSULIN RESISTANCE, NONESTERIFIED FATTY ACIDS AND ADIPOKINES

Dmitry Tanyanskiy[1], E. Firova[1,2], L. Shatilina[2], A. Denisenko[1]

[1]Department of Biochemistry, [2]IEM Clinic, Institute of Experimental Medicine RAMS, St.Petersburg, Russia

Objective: Metabolic syndrome (MS) is a result of complex interaction of different biochemical pathways. The aim was to evaluate the role of insulin resistance, nonesterified fatty acids (NEFA) and adipokines in pathogenesis of atherogenic dislipidemia in patients with metabolic syndrome.

Methods: 113 patients (44 males and 69 females) with MS and 45 patients (24 males and 21 females) without MS were studied. Mean age was 57,4±9,2 years and it didn't differed between groups. All patients had stable forms of coronary heart disease. We measured anthropometric and lipid parameters, concentration of plasma glucose. Insulin, leptin and adiponectin levels were determined by ELISA. Insulin sensitivity was estimated by index HOMA.

Results: Patients with MS in comparison with control group had higher concentrations of triglycerides, NEFA, glucose, insulin, leptin and lower content of high-density lipoprotein cholesterol (HDL-C) and adiponectin. According to multiple linear regression analysis, plasma concentration of triglycerides was determined by body mass index (β=0,40, p< 0,0001), contents of glucose (β=0,22, p=0,003) and NEFA (β=0,23, p=0,003), while concentration of HDL-C was determined only by NEFA content (β=-0,22, p=0,01). Concentration of NEFA was influenced by body mass and HOMA indices, and by adiponectin content (in males). Concentrations of NEFA, leptin and adiponectin were independent determinants of index HOMA.

Conclusions: Basing on this data we suggest that metabolism of NEFA has the main impact on pathogenesis of atherogenic dislipidemia in metabolic syndrome. Influence of insulin resistance and adipokines on forming of dislipidemia is mediated by elevation of NEFA concentration.

Memory Stick

THE FATE OF APO A-I AND APO A-II DURING LIPOSOME-HDL INTERACTION

Marta Czyżewska[1], A. Wolska[1], A. Szutowicz[1], B. Kortas-Stempak[2], M. Wróblewska[1]

[1]Department of Laboratory Medicine, [2]Department of Clinical Chemistry, Medical University of Gdansk, Gdansk, Poland

We have demonstrated that reaction between HDLs and egg yolk lecithin small unilamellar liposomes (SUV) caused the dissociation of apo A-I and apo A-II from HDLs. Here, we investigated the fate of apo A-I and apo A-II during liposome-HDL interaction. At initial SUV-phospholipids (PL)/HDL-PL incubation ratios 1:1, 3:1 and 5:1 HDL released 4.3±1.2, 7.0±1.8 and 13.4±1.9% of apo A-I and 4.4±0.8, 9.9±1.4 and 12.1±2.7 % of apo A-II, respectively. Approximately 51, 49 and 46% of apo A-II and 23, 10 and 15% of apo A-I released from HDLs was bound to non-disrupted liposomes at SUV-PL/HDL-PL 1:1, 3:1 and 5:1, respectively. The remaining part of apolipoproteins generated particles migrating with pre-beta mobility on agarose gel. The PL/apo A-I molar ratios in the pre-beta mobility fractions generated at SUV-PL/HDL-PL 1:1, 3:1 and 5:1were equal to 203.3±47.9, 196.0±43.1 and 183.3±61.5, respectively. Non-denaturing polyacrylamide gel electrophoresis revealed that pre-beta mobility fractions were composed of similar classes of apo A-I particles with diameters of approximately 7.6 and 9.7 nm. At SUV-PL/HDL-PL 1:1 apo A-II generated pre-beta mobility particles with diameters of approximately 7.9 and 9.7 nm, however at higher SUV-PL/HDL-PL ratios additional classes of particles with diameters of 9.1 and 10.7 nm appeared. The generation of pre-beta mobility particles by exchangeable apolipoproteins and liposomes-derived phospholipids may enhance the reverse cholesterol transport. On the other hand, the binding of apo A-II to non-disrupted liposomes may influence their structural stability and lead to loss of apo A-II through hepatic uptake of liposomes.

Memory Stick

STUDY ON THE MOLECULAR BASIS OF FAMILIAL HYPERCHOLESTEROLEMIA IN MEXICO.ADVANCES OF PROJECT

Gerardo Vaca[1], A. Vázquez[1], M.T. Magaña[1], M.L. Ramírez[1], I.P. Dávalos[1], B. Marín[2], E. Martínez[3], G. Carrillo[4]

[1]División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, [2]Departamento de Cardiología, [3]Unidad de Investigación en Epidemiología Clínica, [4]Departamento de Radiología, UMAE-Hospital de Especialidades, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Mexico

Objective: For the reason that:

a) autosomal dominant hypercholesterolemia (ADH) is the most common inherited metabolic disease,

b) the individuals with ADH have a high risk for the development of early onset coronary heart disease, and

c) there are both effective therapeutic options to prevent or reduce the high cardiovascular risk and molecular strategies in order to investigate the genetic cause of ADH.

We implemented a Project aimming to identify families with ADH by cascade screening with purposes of early detection , treatment and genetic counseling as well as to obtain an approximation to the mutational diversity of the ADH in Mexico.

Material and methods: We studied 48 index cases. The clinical and biochemical diagnosis of ADH was defined according to the Guidelines of the International Panel on Management of Familial Hypercholesterolemia. The promotor, the 18 exons and their flanking intronic sequences, of the *LDLR* gene and a portion of the *APOB*

exon 26 were sequenced.

Results: In 25 index cases a total of 19 mutations (LDLR 18, APOB 1) were detected. Among the LDLR mutations:

a) 3 are new (IVS 4-1G-->T; c.1034_1035 ins A ; p.G529D),

b) 5 were observed in two or more probands,

c) the more common was p.C364R (FH-Mexico 3),

d) 2 different mutatios were observed in true homozygote state, and

e) 1 proband had 2 mutations in the same allele and other one was carrier of 2 different mutant alleles.

Finally, by mutational analysis performed in the index cases´ relatives 61 new cases with ADH were detected.

Memory Stick

CRP, LIPIDS AND LIPOPROTEINS AFTER HORMONAL CONTRACEPTION

Vladimir Soska[1], J. Fiala[2], D. Hruba[2], K. Nebeska[2]

[1]Clinical Biochemistry, St. Anna´s University Hospital, [2]Department of Preventive Medicine, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Objectives: The aim of this study was to evaluate the effect of oral hormonal contraception (OHC) on hs-CRP, blood lipids and lipoproteins in young women.

Methods: 44 women (age 22.7±3.5 years, BMI 21.4±2.5, 61 % non-smokers) without any previous hormonal therapy were examined. Total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides (TG), apolipoprotein-B (Apo-B), apolipoprotein-AI (Apo-AI) and C-reactive protein (hs-CRP) were measured before starting OHC and after three months interval. Blood samples were obtained after at least 8 hours of overnight fast. Exclusion criteria were hypertension, dyslipidemia, overweight and obesity.

Results: Significant elevation of CRP level after 3 month of OHC was found (1.06±2.01 vs. 2.34±2.29 mg/L, P< 0.001). There was also significant elevation of TC (4.19±0.80 vs. 4.75±0.79 mmol/L, p< 0.001), LDL-C (2.10±0,64 vs. 2.32±0.66 mmol/L, p< 0.001), HDL-C (1.71±0.42 vs 1.89±0.45 mmol/L, p=0.023), TG (0.85±0.36 vs. 1.18±0.50 mmol/L, p< 0.001), Apo-B (0.58±0.15 vs. 0.69±0.19 g/L, p< 0.001) and Apo-AI (1.55±0.33 vs. 1.88±0.44 g/L, p=0,003). Ratio of both TC/HDL-C and apo-B/apo-AI were not changed.

Conclusion: 3 month usage of oral hormonal contraception has unfavourable effect on hs-CRP level as a markers of cardiovascular risk. On the other hand changes of blood lipids and lipoproteins had no atherogenic character.

Acknowledgements: This study was supported by grant NR 8841-4 of the Grant Agency of the Ministry of Health of the Czech Republic.

Memory Stick

CHOLESTEROL EFFLUX CAPACITY OF MOUSE PERITONEAL MACROPHAGES IS INDEPENDENT OF HUMAN APO A-II EXPRESSION LEVEL AND DIETARY FAT CONTENT

Sira Maïga[1], A. Gaaya[1], L. Fourchaud[1], N. Fournier[2], M. Chabert[1], A. Kalopissis[1]

[1]Centre de Recherche des Cordeliers, UMRS 872, Paris, [2]Laboratoire de Biochimie, EA 3543, Faculté de Pharmacie Châtenay-Malabry, Châtenay-Malabry, France

We aimed to investigate the impact of: the expression level of apo A-II, a major HDL apolipoprotein, and of the lipid content of diets on the cholesterol efflux capacity of mouse peritoneal macrophages (MPM). Wild-type C57BL/6 mice and transgenic mice, either deficient in apo A-II (KO-AII) or expressing human apo AII (hapo A-II) at normal or high levels, were subjected for 2 months to chow, a moderate high-fat (HF) diet (20% by wt coconut oil containing 48% lauric acid) or a very HF diet (49.5% by wt, mostly lard). Thioglycollate-elicited MPM were loaded with cholesterol by incubation with acetyl-LDL and [^{3}H] cholesterol, and treated with cAMP to upregulate ABCA1 expression. Efflux of [^{3}H] cholesterol was measured in the absence or presence of acceptors (lipid-free human apo A-I or apo A-II in equimolar concentrations). Nascent HDLs were detected in incubation media by bi-dimensional electrophoresis.

MPM from all groups of mice displayed similar cholesterol efflux capacities, both in the absence of acceptors (passive efflux) and in the presence of apo A-I or apo A-II (ABCA1-mediated efflux). Of note, apo A-I and apo A-II promoted cholesterol efflux to similar extents. Nascent HDLs were formed with apo A-I and apo A-II.

In conclusion, MPM prepared from mice with various expression levels of hapo A-II and adapted to chow or HF diets maintained cholesterol efflux capacities comparable to those of wild-type and KO-AII mice. Moreover, similar amounts of nascent HDLs were formed by all groups of MPM.

Memory Stick

DECREASE OF PLASMA LEVELS OF LIPOPROTEIN A [LP(A)] AFTER ADMINISTRATION OF L-CARNITINE

Konstantinos Gkogkos, O. Nikitidou, E. Panara, O. Siarkou, C. Miggos, T. Plougarlis, G. Pavlidis

2nd Internal Medicine Department, General Hospital "G. Papanikolaou", Thessaloníki, Greece

Background and aims: Lipoprotein a [Lp(a)], according to multicenter randomized studies, has been identified as an independent risk factor for cardiovascular disease and a prognostic index of atherosclerosis. The aim of the present study was to determine the influence of L-carnitine administration in patients with hyper Lp(a).

Patients and methods: 79 patients (47 women and 32 men, 59.5% και 40.5% respectively) with mean age±sd 53±17 years participated in the study. They were all recruited from the Hypertension office and the Lipid office of an Internal Medicine Department. All of them had increased levels of Lp(a) (38-74 mgr/dl). L-carnitine was administered to them in a dose of 2 gr per day without modification of their prior pharmacological treatment. We assessed fasting plasma levels of Lp(a) and total lipid profile at baseline and 3 months after initiation of treatment with L-carnitine.

Results: Our findings was that Lp(a) levels decreased by an average of 6.1% in the patients who received L-carnitine supplementation over a period of 3 months. All the other biochemical parameters of their lipid profile, namely serum total cholesterol, LDL cholesterol, non-HDL cholesterol and triglycerides, were not significantly affected.

Conclusion: According to the results of the present study, dietary supplementation of L-carnitine seems to have an important role in the management of patients with increased levels of Lp(a) and could be used in order to protect them against cardiovascular morbidity and mortality.

Memory Stick

THE IMPACT OF EZETIMIBE ADMINISTRATION ON THE LIPID PROFILE OF PATIENTS WITH PRIMARY DYSLIPIDEMIA

Konstantinos Gkogkos, O. Siarkou, O. Nikitidou, E. Panara, C. Miggos, T. Plougarlis, G. Pavlidis

2nd Internal Medicine Department, General Hospital "G. Papanikolaou", Thessaloníki, Greece

Background and aims: Primary dyslipidemia is believed to be associated with increased risk for cardiovascular disease. The aim of the present study was to evaluate the effect of ezetimibe administration on the lipid profile of patients with primary dyslipidemia who could not tolerate statin therapy or could not achieve the therapeutic goals with statin monotherapy.

Patients and methods: We studied the administration of ezetimibe (10 mgr/day) for 6 months in 160 patients with primary dyslipidemia, aged 44-82 years. They were all recruited from the Hypertension office and the Lipid office of an Internal Medicine Department. 92 of all the patients, who participated in the study, had not achieved their recommended therapeutic goals with statin monotherapy (group 1), while 68 could not tolerate statin therapy (group 2). The fasting plasma concentrations of total cholesterol, LDL cholesterol, non-HDL cholesterol and triglycerides at baseline and after 6 months of ezetimibe treatment were measured in both groups.

Results: We observed in group 1 decrease in the levels of serum total cholesterol by 18%, LDL cholesterol by 19%, non-HDL cholesterol by 17.5% and triglycerides by 10%. Respectively in group 2 decrease was observed in the levels of serum total cholesterol by 16%, LDL cholesterol by 17%, non-HDL cholesterol by 14% and triglycerides by 8%.

Conclusion: Ezetimibe administration has a favorable impact on the lipid profile of patients with primary dyslipidemia, who had not achieved the therapeutic goals with statin monotherapy or could not tolerate statin therapy, decreasing the risk associated with cardiovascular disease.

Memory Stick

RELM-A REDUCES ATHEROSCLEROSIS VIA REGULATION OF LIPID METABOLISM

You-Han Lee, M.-R. Lee, M. Cho, G.T. Oh

Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul, Republic of Korea

Resistin-like molecules (Relm)-α is a cytokine that belongs to a family of proteins including resistin, relm-β and relm-γ and is a highly expressed in adipose tissue. Recent studies showed that these proteins control glucose metabolism and especially, adipokine resistin play important roles in various metabolic syndromes such as obesity, diabetes and atherosclerosis. Nevertheless, the effects of relm-α on atherosclerosis still remain unknown. In this study, we sought to characterize the role of relm-α in the pathogenesis of atherosclerosis by generating relm-α-overexpressing mice. To search regulatory molecules by relm-α on hepatic metabolism, cDNA microarray was carried out in liver of normal chow or western diet-fed relm-α-overexpressing mice compared to control animals and the alterations of various factors involved in lipid metabolism were identified. Validation of microarray data using RT-PCR and real-time PCR showed up-regulation of ABCA1 gene and down-regulation of VLDL receptor, PPARγ and HMG-CoA synthase. Moreover, overexpression of relm-α significantly decreased plasma cholesterol levels. These findings suggest that relm-α may reduce atherosclerosis via regulation of lipid metabolism.

Memory Stick

SINGLE DOSES OF 2 FORMULATIONS OF PITAVASTATIN SHOW EQUIVALENT PHARMACOKINETICS IN HEALTHY EUROPID AND JAPANESE MEN

Neil Hounslow[1], S. Nakagawa[1], S. Warrington[2]

[1]Kowa Research Europe Ltd, Wokingham, [2]Hammersmith Medicines Research, London, UK

Pitavastatin (PIT) is a HMG-CoA reductase inhibitor marketed in Japan (J) as tablets containing 1 and 2mg PIT as the calcium salt. The investigational European (E) tablets contain 1, 2 and 4mg PIT as the base. The dose of active PIT in the two formulations differs by 5%. This two-way crossover, open label study aimed to identify potential pharmacogenomic differences between Europid and Japanese populations by comparing the pharmacokinetics of single 2mg doses of the two PIT formulations in healthy Europid (n=48) and Japanese (n=12) men aged 18-45 years. After both formulations, the median T_{max} for PIT and PIT's lactone metabolite (PL) was 1h (range: 0.5-2h) and 1.5h (range: 1-3h) respectively. Mean values and variability of C_{max} and AUC_{0-t} of PIT and PL for both formulations were equivalent. The ratio of the least-square geometric means (LSGM) of PIT in Europid subjects comparing the E and J formulations were 103.1 and 99.2 respectively (90% confidence limits [CL] within 80-125% for both PIT and PL).

The PIT C_{max} and AUC_{0-t} for the E formulation in Europid subjects were slightly lower than for the J formulation in Japanese subjects (ratio of LSGMs 92.6 and 87.9 respectively) but PL values were equivalent. Adjustment for age and weight produced equivalent values for PIT 101.5 (90% CL 89.6-115.1) and 96.7 (90% CL 85.6-109.3), respectively, and for PL.

In conclusion, the J and E formulations of PIT are bioequivalent and show no pharmacogenomic differences between Europid and Japanese men.

Memory Stick

HORMONOTHERAPY AND LIPIDS IN POSTMENOPAUSAL WOMEN WITH BREAST CANCER

Christodoulos Kypridemos, K. Rossios, K. Traianou, C. Dontas, F. Lagra, X. Krokidis, S. Papantoniou, P. Papadopoulou-Zekeridou

First Department of Internal Medicine, General Hospital of Kavala, Kavala, Greece

Background: The inhibition of estrogens is a very important element, in breast cancer therapy with positive ER and PR receptors. Our study is aiming to investigate the relation of hormonotherapy and lipids in postmenopausal woman with breast cancer.

Methods: 39 patients were studied (mean age 67,4) treated with tamoxifen n=16, letrozole n=18 and exemestane n=5. Blood samples were collected in the beginning of the hormonotherapy and 6 months later. The total cholesterol and triglycerides levels were determined. Subjects were not treated with statins and the Student's t test for pairs was used.

Results: In the tamoxifen group TC had no change and TG an insignificant increase (p=0,09). In the letrozole group an insignificant increase of TC (p=0,12) and no alteration of TG levels was observed. Finally, in exemestane group the TC was significantly increased (p=0,01) while the TG was increased but not statistically signifigant (p=0,13).

Conclusion: All three drugs alter the lipid profile of patience but only the exemestane causes a significant change.

The better prognosis of patients treated with aromatase inhibitors (than tamoxifen) may be related to the increase of lipid serum levels, by decreasing neo-angiogenesis. More studies are needed to balance the cardiac risk and the metastasis risk on breast cancer.

Memory Stick

MONITORING OF PLATELET FUNCTION AND EICOSANOID RELEASE USING LC-MS/MS IN PATIENTS WITH PHENYLKETONURIA (PKU)

U. Mütze[1], S. Beblo[1], **Uta Ceglarek**[2], M. Brügel[2], L. Kortz[2], G.M. Fiedler[2], A. Leichtle[2], J. Thiery[2], W. Kiess[1]

[1]University Children`s Hospital, [2]Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Universität Leipzig, Leipzig, Germany

Patients with phenylketonuria (PKU) need a protein restricted diet. This results in an unbalanced nutrition with essential fatty acids, since phenylalanine free amino acid mixtures do not contain lipids. This long-term dietary restriction of polyunsaturated fatty acids may interfere with platelet and leukocyte functions in this patients.

Eicosanoids are lipid mediators that are oxidized from long chain polyunsaturated fatty acids (LC-PUFA), primarily arachidonic acid by enzymatic or non-enzymatic peroxidation of cell membrane-bound phospholipids. Eicosanoids are essential mediators in inflammation and haemostaseological reactions. The eicosanoid thromboxane A2 (TXA_2) is important for platelet activation and vasoconstriction. Therefore, analysis of platelet activation could help understanding the influence of imbalanced LC-PUFA intake on hemostaseological processes in patients with PKU.

We developed a novel analytical protocol for the characterization of platelet activation and eicosanoid release. Platelet activation in platelet-rich human plasma was induced with ADP, collagen, or arachidonic acid and analyzed by aggregometry. The following eicosanoid production was measured by multiparametric liquid chromatography- tandem mass spectrometry (API 4000).

Platelet activation with collagen and arachidonic acid resulted in a marked production of eicosanoid metabolites of the cyclooxygenase and lipooxygenase pathway. However, ADP activation did not induce measurable eicosanoid releases. In our pilot study we will present first results from children with PKU and healthy controls.

Memory Stick

MOLECULAR STUDY OF *LPL*, *APOAIV*, *APOAV*, *APOCII* AND *APOCIII* GENES IN PATIENTS WITH HYPERTRIGLYCERIDEMIA

Tânia Santos[1], A. Gaspar[2], I.M. Gaspar[3], Q. Rato[4], M. Bourbon[1]

[1]Dpt. de Promoção da Saúde e Doenças Crónicas, Instituto Nacional de Saúde Dr. Ricardo Jorge, [2]Dpt. de Doenças Metabólicas (Pediatria), Hospital Sta. Maria, [3]Dpt. de Genética Médica, Hospital Egas Moniz, Lisboa, [4]Dpt. de Cardiologia, Hospital S. Bernardo, Setúbal, Portugal

The purpose of this study is the identification of genetic alterations responsible for hypertriglyceridemia (HTG) in patients with Familial Lipoprotein Lipase Deficiency (FLLD) and Familial Hypertriglyceridemia (FHT) phenotypes.

A genetic analysis was performed in 17 patients: 3 with FLLD phenotype and 14 with FHT. *LPL* and apolipoproteins genes (*APOAIV, APOAV, APOCII, APOCIII*) were amplified by PCR followed by direct sequencing. Study of large rearrangements in *LPL* was done by MLPA.

One patient with FLLD phenotype was homozygote for *APOCII* Y37D. FHT patients showed functional alterations in *LPL* (N291S, 2 patients; N291S/G188E, 1 patient; -93T>G/D9N/S447X, 1 patient), *APOAIV* (Q360H, 1 patient; T347S, 2 patients), *APOAV* (S19W, 1 patient) and *APOCIII* (3238G>C, 2 patients; 3238G>G, 2 patients). *APOCII* Y37D was not described before. There were no large rearrangements in *LPL* detected by MLPA.

According to these results, both heterozygote alterations in *LPL* and alterations in apolipoproteins genes seem to influence serum triglycerides in Portuguese FHT patients. One patient with clinical diagnosis of FLLD had Familial Apolipoprotein C-II Deficiency instead, which shows the importance of molecular study of *LPL* and *APOCII* in FLLD. Both FHT and FLLD are characterized by HTG and since HTG is an independent risk factor for cardiovascular disease (CVD), it is extremely important to identify affected patients and relatives in order to prevent the occurrence of premature CVD in these patients.

Memory Stick

LIPOPROTEIN FILTRATION BY MONET: FIRST CLINICAL APPLICATION IN LDL REMOVAL

Jens Ringel

Dialyse- und Apheresezentrum Potsdam-Babelsberg, Potsdam, Germany

Introduction: LDL-apheresis is used to lower LDL-cholesterol especially in patients suffering from familial hypercholesterolaemia when medication is not enough. We report about the first experiences with a new lipoprotein filtration system, MONET.

Aim: To show the efficacy and safety of the new lipoprotein filtration system MONET

Methods: We treated five symptomatic hypercholesterolaemic atherosclerotic patients on a weekly basis with the new MONET membrane filter (Fresenius Polysulfon). All patients received maximum drug therapy. Each patient's single plasma volume was treated per session. A heparin bolus of 5.000 IU was given at the beginning and continuous citrate anticoagulation performed at a v/v ratio of 1:30 of blood flow. Blood flow was 80 ml/min and plasma flow was 24 ml/min.

Results: 40 treatments were performed and on average 3650 ml plasma treated. Clinical tolerance was good and technical performance of the system without any problems. Mean reduction per treatment was: total cholesterol 51,4 ± 6,5 %, triglycerides 39,1 ± 18,3 % , LDL-C 67,8 ± 9,5 % (144,2 à 46,7 mg/dl) and HDL-C cholesterol 20,5 ± 8,0 %. Total protein loss was 17,6 ± 3,3 % and albumin loss 12,9 ± 4,1 %. The majority of side effects were apheresis related hypotension (2,5 %). One hypocalcaemic episode occurred due to citrate anticoagulation and one blood access problem.

Memory Stick

LIVER-SPECIFIC LIPOPLEXES FOR THE KNOCKDOWN OF SCAVENGER RECEPTOR CLASS B TYPE I

Kristina Duwensee, I. Tancevski, E. Demetz, P. Eller, C. Heim, E. Huber, A. Schroll, A. Ritsch, J. Patsch

Department of Internal Medicine I, Medical University Innsbruck, Innsbruck, Austria

Introduction: Scavenger receptor class B type I (SR-BI) is known to mediate the bidirectional flux of cholesterol across the plasma membrane. Recently, it became clear that SR-BI also mediates the entry of bacteria into different cell types. Additional studies revealed that crucial role of hepatic SR-BI in plasmodium infection, and may therefore constitute a good target for malaria prophylaxis. We aimed to construct non-viral vectors for hepatic delivery of siRNA specific for SR-BI.

Methods and results: We designed siRNA specific for both, murine and human SR-BI and tested the cellular uptake of siRNA by several transfection constructs including polyethylenimine, chitosan, liposomes or mixtures of these substances. Using different cell lines (J774, FL83, HepG2) as well as primary macrophages and hepatocytes, transfection was monitored by fluorescently labeled siRNA and analysed by western blot. Transfection was shown to be most efficient when siRNA was protected from degradation by a defined mixture of polyethylenimine and lipids. Moreover, transfection rates crucially depended on size of the lipoplexes and was highest using constructs with a diameter of 100nm. Employing these lipoplexes, we were able to knockdown SR-BI by 50% in macrophages and 85% in hepatocytes. Performing a radioactive HDL-uptake in J774, we showed also a knockdown in SR-BI functionality (45%).

Conclusion: These lipoplexes as a vector for SR-BI-specific siRNA might represent a new tool for the intervention against hepatic infections. Future experiments in mice are heavily awaited to test whether this novel gene therapeutic approach will protect from bacterial infection in vivo.

Memory Stick

A NEW METHOD TO FIND OUT THE HYDROPHILITY OF SERUM-LIPOPROTEINS

Günter Schrot

Johanniter Krankenhaus Treuenbrietzen GmbH, Treuenbrietzen, Germany

Introduction: Serum lipoproteins are micellar structured particles with a hydrophobic core and a hydrated hydrophilic surface, caused by the dipolar character of water molecules. The structure of vascular endothelium surface is similar: a hydrated layer with hydrophilic parts inside and hydrophobic parts outside the blood stream. Based on the second thermodynamic law, the duration of contacts between lipoproteins and the endothelium is prolonged at lower hydrophilic lipoproteins.

Methods: Serum lipoproteins are incubated with a micellar indicator, a nonylphenol ethoxylate, which hydrophility ist known. Formation of mixed micells were following. The optical behavior (cloud point) of these mixed micells is changing depending on the hydrophility of lipoproteins. The hydrophility of lipoproteins from 15 patients with peripher occlusiv arterial disease and 96 healthy persons were determined.

Results: A micellar indicator was found to determine the hydrophility of lipoproteins. In relation to the concentrations of cholesterols, triglycerids and phospholipids the hydrophility of lipoproteins from patients with peripher occlusive arterial disease was significantly lower than these of healthy persons.

Conclusions: In theory the thermodynamic interactions between lipoproteins and the endothelium surface of the vessel wall can be prolonged by hydrophobic lipoproteins. Thus, there is a high possibility of more receptor contacts and more deposits of cholesterol and triglycerids. This may contribute to the pathogenesis of atherosclerosis. Further studies must show if a lower hydrophility of serum lipoproteins is a pathogenetic factor of atherosclerosis.

Memory Stick

LIPID PROFILE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION ATTENDED IN OUR HOSPITAL

Antoni Gavarró, R. Tomas, G. Celma, M. Martinez, Y. Sanchez

Laboratory, UDIAT-CD Corporació Parc Tauli, Sabadell, Spain

Introduction: It is well known that Dyslipemia is one of the most common risk factors for coronary artery disease (CAD).

Objective: The aim of this study is to evaluate the lipid profile in a group of patients affected of acute myocardial infarction (AMI) and attended in our Hospital.

Material and methods: This study was performed in a group of 220 patients (120 male) with AMI and 410 healthy individuals regarded as controls.

AMI was confirmed by ECG changes and cardiac enzymes. Lipid profile included Total Cholesterol, LDL-Cholesterol, HDL-Cholesterol and Tryglicerides, all measured in a Modular PPE autoanalyzer (Roche Diagnostics).

Results: Patients with AMI showed a higher mean LDL-Cholesterol, LDL-C/HDL-C ratio, and T.C/HDL-C ratio compared to controls, but a lower HDL-C and Trygliceride serum levels.

No significant differences were found in total Cholesterol serum levels between patients and healthy controls.

Conclusion: We can suggest with these results that Hyperlipidemia behaves as an important predictor for Coronary artery disease.

Memory Stick

DIFFERENTIAL SORTING OF APOER2 AND VLDL RECEPTOR AND IMPLICATIONS TO THE REELIN SIGNALING PATHWAY

Johannes Nimpf

Medical Biochemistry, Medical University of Vienna/MFPL, Vienna, Austria

ApoER2 and VLDL receptor bind Reelin and transmit the signal into migrating neurons of the central nervous system. To a certain extent, both receptors can compensate for each other, and only the loss of both receptors results in the *reeler* phenotype which is characterized by a gross defect in the architecture of laminated brain structures. Yet, both receptors also have specific distinct functions, as corroborated by analyses of the subtle phenotypes displayed in mice lacking either ApoER2 or VLDL receptor. The differences in their function(s), however, have not been defined at the cellular level.

The aim of this study was to define the molecular basis of differential sorting of ApoER2 to raft and VLDL receptor to non-raft domains of the plasma membrane and link their specific sorting to functional differences of the receptors.

VLDL receptor mediates rapid endocytosis of Reelin and destines Reelin for degradation via the clathrin-coated pit - clathrin-coated vesicle - endosome pathway without being degraded to a significant extent. Binding of Reelin to ApoER2, a resident of rafts leads to the production of specific receptor fragments with specific functions of their own and to degradation of ApoER2 via lysosomes. These features contribute to a receptor-specific fine-tuning of the Reelin signal, leading to a novel model which emphasizes negative feedback loops specifically mediated by ApoER2 and VLDL receptor, respectively.

Memory Stick

LIPID PEROXIDATION WAS REDUCED BY AEROBIC TRAINING IN WOMEN WITH METABOLIC SYNDROME

Manuel Rosety-Rodriguez[1], G. Fornieles-Gonzalez[1], I. Rosety[2], M.A. Rosety[2], N. Garcia-Gomez[3], A. Camacho[4], F.J. Ordoñez[2]

[1]Internal Medicine, [2]School of Sport Medicine, [3]Pathologic Anatomy, University of Cadiz, Cadiz, [4]Medicine, Hospital Juan Ramon Jimenez, Huelva, Spain

Background and aims: Oxidative stress is one of the most important factors in the etiopathogenesis of metabolic syndrome. Fortunately we have recently found aerobic training at low/moderate intensity improved antioxidant defense system in patients with metabolic syndrome. For the reasons already mentioned the present study was designed to determine the influence of regular exercise on lipid peroxidation in women with metabolic syndrome.

Methods: Sixty young women with metabolic syndrome according to the criteria reported by the National Cholesterol Education Program Adult Treatment Panel III volunteered for this study. Fourty five were randomly included in experimental group to perform a 12-week aerobic training program, 3 days/week, consisting of warm up (10 min), main part (20-35 min [increasing 5 minutes each three weeks]) at a work intensity of 60-75% of peak heart rate (increasing 5% each three weeks) and cool-down (10 min). Control group included 15 age, sex and BMI-matched women with metabolic syndrome that will not perform any training program. Written informed consent was obtained from all participants. Further our protocol was approved by an institutional ethic committee. Plasmatic malondialdehyde (MDA) levels were assessed using the method of Draper and Hadley twice: 72-hours before starting the program (pre-test) and after its ending (post-test).

Results: When compared to baseline, malondialdehyde (MDA) levels were decreased significantly after being exercised (1.22±0.19 vs 0.86±0.15 nmol/ml; $p < 0.001$). On the contrary no changes were reported in controls.

Conclusions: It may be concluded a 12-week aerobic training program reduced plasmatic malondialdehyde in women with metabolic syndrome.

Memory Stick

HORMONAL-METABOLIC ASPECTS OF HYPERLIPIDAEMIAS IN NON-OBESE TYPE II DIABETIC PATIENTS WITH ATHEROSCLEROSIS

Vasile Anestiadi[1], V. Anestiadis[1], Z. Anestiadi[2]

[1]Center for Pathobiology and Pathology, Academy of Sciences, [2]Chair of Endocrinology, Medical University, Chisinau, Moldova

Objective: The aim was to evaluate the hormonal-metabolic aspects of atherogenic dyslipidaemias in non-obese patients (pts) with type II diabetes mellitus (DM) and atherosclerosis (ATH).

Methods: Group 1 (gr1) was composed of 30 normals (age = 54.50-2.03; means-SEM). Group 2 (gr2) consisted of 20 non-obese type II DM pts with hypertriglyceridaemia (type IV HLP) and ATH (age = 53.19-1.91). Group 3 (gr3) consisted of 23 non-obese type II DM pts with mixed hyperlipidaemia (type IIB HLP) and ATH (age = 56.73-1.96). Following were determined in serum, in fasting state: total cholesterol (CH), HDL-cholesterol (HDL-CH), atherogenicity coefficient (HAC), triglycerides (TG), lipolytic activity (LA), lipoprotein fractions, prostaglandins A1 and E1, prostaglandin F2alpha (PGF). Following were determined in plasma, during standard OGTT: glucose, insulin, insulin/glucose index (IGI), glucagon, C-peptide, STH, somatostatin, ACTH, cortisol, aldosterone, beta-endorphin.

Results: Both gr2 and gr3 pts, compared to gr1, had higher body mass, CH, TG, HAC, and lower HDL-CH, LA, insulin (at OGTT hour 1), IGI, STH (hour 2), basal aldosterone. Gr2 pts, compared to gr1, had lower STH (hours 1 and 2). Gr3 pts, compared to gr1, had higher glucagon (hour 2), somatostatin (hours 0 and 1), cortisol (hours 1 and 2), PGF, and lower C-peptide (hour 1), STH (hours 0 and 2).

Conclusions: Altered hormonal-metabolic patterns were observed in non-obese type II DM pts with ATH and dyslipidaemias, including decreased STH and elevated cortisol.

Memory Stick

AGE-RELATED ELEVATED LEVEL OF C-TERMINAL TRUNCATED APOLIPOPROTEIN A-I IN HIGH-DENSITY LIPOPROTEIN WITH AN INCREASE OF CHOLESTERYL ESTER TRANSFER ACTIVITY

Ki-Hoon Park[1], W. Jang[1], D.-G. Shin[2], J.-R. Kim[3], K.-H. Cho[1]

[1]School of Biotechnology/ Aging-associated Vascular Disease Research Center/ Yeungnam University, Gyeongsan, [2]Cardiovascular Division/ Internal Medicine/ Yeungnam University Medical Center, [3]Department of Biochemistry and Molecular Biology/ Aging-associated Vascular Disease Research Center/ Yeungnam University, Daegu, Republic of Korea

In order to compare the change in lipoprotein metabolism with aging, we analyzed the lipid and protein compositions of individual lipoprotein fractions, which were purified from elderly male subjects, with young subjects serving as controls.

Healthy and non-obese elderly subjects (OH group, n=16) had a normal range of serum total cholesterol (TC), triacylglycerol (TG), and high-density lipoprotein-cholesterol (HDL-C), although slightly higher than in young subjects (control group, n=10). However, the OH group had a 9-fold increased serum high sensitivity C-reactive protein and a 2-fold increased serum uric acid level. The OH group had a 2-fold stronger cholesteryl ester transfer protein (CETP) activity in the HDL_3 fraction, even though the OH-group had a loss of serum antioxidant ability. Both groups had no differences in serum parameters of heptatic inflammation, and interleukin-6 and glucose levels. ApoA-I levels in serum and lipoprotein-deficient serum were 1.5-fold increased in the OH group by enzyme linked immunosorbent assay, indicating an age-related increase in lipid-free apoA-I. Conclusively, C-terminal truncation of apoA-I, loss of antioxidant activity, and an increase in HDL_3-CETP activity were correlated with aging.

Running head: Age-related increase of lipid-free apoA-I

Memory Stick

FEMALES WITH ANGINA PECTORIS HAVE ALTERED LIPOPROTEIN METABOLISM WITH IMPAIRED HDL-ASSOCIATED ANTIOXIDANT ENZYMES

Ji-Hye Lee, S.-M. Kim, K.-H. Cho

School of Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea

In order to investigate non-invasive biomarkers for angina pectoris (AP), we analyzed the lipid and protein composition in individual lipoproteins from females with angina pectoris (n=12) and age- and gender-matched controls (n=10). In the low-density lipoprotein (LDL) fraction, the triglycerides (TG) and protein was increased in the AP group compared to the control group. The AP group had lower total cholesterol (TC) and elevated TG in high-density lipoprotein (HDL). In the AP group, cholesteryl ester transfer protein (CETP) activity was enhanced in HDL and LDL, while lecithin:cholesterol acyltransferase (LCAT) activity in HDL_3 was almost depleted. Antioxidant activity was significantly decreased in the HDL_3 fraction, with a decrease in the HDL_2 particle size. In the HDL_3 fraction, paraoxonase and platelet activating factor-acetylhydrolase (PAF-AH) activity were much lower and the levels of CETP and apoC-III were elevated in the AP group. The LDL from the AP group was more sensitive to cupric ion-mediated oxidation with faster mobility. Thus, the lipoprotein fractions in the AP group had impaired antioxidant activity and increased TG and apoC-III with structural and functional changes.

Memory Stick

MECHANISM OF HYPERCHOLESTEROLEMIA IN PRAGUE HEREDITARY HYPERCHOLESTEROLEMIC (PHHC) RAT

Miluse Zimolova[1,2], M. Coufalikova[1,2], M. Heczkova[1], R. Bohuslavova[1,2], V. Stranecky[3], R. Ivanek[4], M. Jirsa[1], R. Poledne[1,2], J. Kovar[1,2]

[1]Institute for Clinical and Experimental Medicine, [2]Centre of Cardiovascular Research, [3]Institute of Inherited Metabolic Disorders, 1st Medical Faculty, Charles University, [4]Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic

Prague Hereditary Hypercholesterolemic (PHHC) rat develops hypercholesterolemia after cholesterol feeding. The hypercholesterolemia is polygenic and characterized by an accumulation of cholesterol in lipoproteins other than HDL, mostly in VLDL. However, the exact mechanism responsible for development of hypercholesterolemia has not been characterized yet.

Objectives: To characterize this mechanism we studied VLDL production rate in PHHC rats. In a separate experiment we carried out the analysis of transcriptome in the liver.

Methods: Male PHHC and Wistar rats were fed chow (C) or 1% cholesterol (CHOL) diet for three weeks. The VLDL concentration was measured 2 hours after i.v. application of Triton WR 1339. Hepatic gene expression was evaluated using Affymetrix GeneChip arrays.

Results: On CHOL diet, cholesterol and triglycerides accumulated in the liver of both PHHC and Wistar rats whereas cholesterolemia rose significantly only in PHHC rats. In response to CHOL diet PHHC rats produce VLDL that do not differ in TG content but carry twice more cholesterol than VLDL of Wistar rats. However, the analysis of transcriptome did not reveal any differences in the response of gene expression to CHOL diet between both strains. On the other hand, several genes without any known role in lipoprotein metabolism were expressed differently between PHHC and Wistar rats (*Aldh1a7, Yc2, Apof, Ugt2b, Cdh17, Ltc4s,* and *Slc6a6*).

Conclusions: The production of cholesterol-enriched VLDL may play a role in development of hypercholesterolemia, but metabolic pathways linking their production to gene expression remain to be determined.

Supported by grant 1M0510 from MEYS CR.

Memory Stick

ADIPOCYTE FACTORS, HSCRP AND MALONDIALDEHYDE LEVELS IN OVERWEIGHT POSTMENOPAUSAL WOMEN WITH NORMAL AND IMPAIRED OGTT

E. Kassi[1], G. Hroussalas[1], M. Dalamaga[1], K. Kazanis[1], G. Merantzi[2], A. Zachari[3], E.J. Giamarellos-Bourboulis[4], **Amalia Dionyssiou-Asteriou**[1,3]

[1]Department of Clinical Biochemistry, Medical School, University of Athens, [2]Laboratory of Biochemistry, Evangelismos General Hospital, Athens, [3]Department of Biological Chemistry, Medical School, University of Athens, [4]4th Department of Internal Medicine, Medical School, University of Athens, Athens, Greece

Background: Menopause is related to events contributing to atherosclerosis progression and cardiovascular disease development, such as weight gain, lipid metabolism alterations, insulin resistance, oxidative stress and low-grade inflammation. In overweight/obese postmenopausal women we investigated the association of circulating levels of atherogenic and antiatherogenic adipocyte factors, leptin and adiponectin respectively, with high sensitivity C-reactive protein (hsCRP) and malondialdehyde (MDA) in relation to impaired glucose tolerance (IGT).

Methods: Thirty eight overweight/obese postmenopausal women, age 48-69 years, were included. Eighteen with normal glucose metabolism (NGT), BMI 28-32, waist circumference (WC) 82-104 cm and twenty with IGT (diagnosed by OGTT), BMI 29-40, WC 87-115 cm. Leptin and adiponectin were evaluated by ELISA at 0 and 120 min of OGTT, hsCRP and MDA were also measured at 0 and 120 min. Serum glucose, insulin, total cholesterol, HDL and hsCRP were measured in automated analyzer (Roche Diagnostics). MDA was measured by HPLC. Insulin resistance (HOMA)/sensitivity (QUICKI) indexes were estimated.

Results: In subjects with NGT, hsCRP levels were positively correlated with fasting leptin ($p=0.03$) and HOMA ($p=0.004$), while in subjects with IGT negatively with QUICKI ($p=0.001$). In both groups, hsCRP levels were positively correlated with fasting insulin ($p< 0.03$) and with BMI and WC ($p< 0.02$), while fasting adiponectin with HDL ($p< 0.01$). During OGTT there was a significant increase ($p< 0.001$) of leptin and MDA levels in both groups.

Conclusion: There is a relationship between obesity, insulin and sub-clinical inflammation. Leptin levels and lipid peroxidation are linked to hyperglycaemic state. Adiponectin could exert its antiatherogenic effect through HDL.

Memory Stick

PCSK9: FROM GENE AND VARIANTS TO PROTEIN AND PLASMA LEVELS

Marianne AbiFadel[1,2], J.-P. Rabès[1,3], M. Varret[1], M.-E. Samson- Bouma[1], E. Bruckert[4], M. Devillers[1], V. Carreau[4], L. Tosolini[1], M. Marduel[1], A. Marques[1], P. Couvert[4], D. Bonnefont-Rousselot[5], M. Guérin[4], A. Carrié[4], A. Prat[6], N. Seidah[6], C. Boileau[1,3]

[1]INSERM U781, Hôpital Necker, Université Paris Descartes, Paris, France, [2]Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon, [3]Laboratoire de Biochimie et de Génétique Moléculaire, Hôpital Ambroise-Paré, APHP; Université Versailles-Saint-Quentin-en-Yvelines, UFR Médicale Paris-Ile-de-France-Ouest, Boulogne, [4]INSERM UMR S939, Hôpital Pitié-Salpêtrière, [5]UF de Biochimie des Maladies Métaboliques, Hôpital Pitié-Salpêtrière, APHP, Paris, France, [6]Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, QC, Canada

Introduction: Hypercholesterolemia is one of the major causes of coronary heart disease (CHD). The genes encoding the low density lipoprotein receptor (*LDLR*) and its ligand apolipoprotein B (*APOB*) have been the two genes classically implicated in autosomal dominant hypercholesterolemia (ADH). Our discovery of the implication of *PCSK9* in hypercholesterolemia by linkage analysis studies in ADH families with no mutation in the *LDLR* or in the *APOB* genes brought to light an unknown actor in cholesterol metabolism that has been extensively investigated since. Several *PCSK9* variants have been identified, some of them are gain of function mutations causing hypercholesterolemia by a reduction of LDL receptor levels, while others are loss of function variants associated with a reduction of LDL-C levels and a decrease in the risk of CHD. PCSK9 is a secreted protein that represents now a major therapeutic target for the treatment of hypercholesterolemia and prevention of CHD.

Methods and results: By studying further patients with hypercholesterolemia unrelated to the *LDLR* or the *APOB* genes, we have recently identified new variants of *PCSK9* and studied the effect of these variants on the maturation of the enzyme. Furthermore, we measured PCSK9 concentration, by an ELISA method, in the plasma of patients with familial hypercholesterolemia carrying different mutations of *PCSK9* especially the p.S127R, p.F216L, p.R218S mutations that we have already reported.

Conclusions: These data further contribute to the characterization of *PCSK9* variants and mutations and their impact in cholesterol diseases.

Memory Stick

ANTI OX-LDL ANTIBODIES CORRELATE WITH SOLUBLE INTERLEUKIN 2 RECEPTOR IN PATIENTS WITH CLINICALLY DIAGNOSED FAMILIAL HYPERCHOLESTEROLEMIA (FH)

Malgorzata Walus-Miarka[1], A. Hubalewska-Dydejczyk[2], D. Fedak[3], M. Opalinska[2], T. Stompor[4,5], P. Miarka[4], D. Czarnecka[6], B. Idzior-Walus[1]

[1]Department of Metabolic Diseases, [2]Department of Endocrinology, [3]Clinical Biochemistry, [4]Department of Nephrology, Jagiellonian University Collegium Medicum, Krakow, [5]Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury, Olsztyn, [6]Department of Cardiology, Jagiellonian University Collegium Medicum, Krakow, Poland

Higher level of autoantibodies against oxidized LDL (anti-ox-LDL) was suggested as a marker of atherosclerosis. Elevation of soluble interleukin-2 receptor (sIL2r) give in vivo evidence of T-cell activation in the atherosclerotic process. The aim of this study was to explore associations between plasma sIL2r levels and oxidized LDL (ox-LDL), anti-ox-LDL and some inflammation markers in patients with familial hypercholesterolemia.

Material included 28 patients with familial hypercholesterolemia (diagnosis based on Simon Broome criteria) and advanced atherosclerosis, confirmed by measurement of carotid IMT. Plasma lipids were determined enzymatically, sIL2r,

s-ICAM, ox-LDL, IL-6, anti-ox-LDL concentrations by ELISA, serum amyloid A (SAA) and hs-CRP using nephelometry. Mean age of patients (10M,18F) was 52,9±10 years. The mean values of total, LDL, HDL-cholesterol and triglyceride concentrations were as follows: 5.25±1.2; 3.7±1.3; 1.5±0.5 and 1.4±0.7 mmol/l. Mean value of sIL2r was 1068.3±436.4 pg/ml. Anti-ox-LDL serum concentration equaled 359.4±408 mU/mL, median 162.2 mU/mL and ox-LDL 100.91±95.06 ng/mL, median 102 ng/ml. The plasma level of sIL2r positively correlated with s-ICAM ($r=0.46$, $p=0.01$), ox-LDL ($r=0.59$, $p=0.0008$) and anti-ox-LDL ($r=0.47$, $p=0.01$). No correlations between sIL2r and other inflammation markers were found. Multiple regression analysis with plasma level of sIL2r as dependent variable and anti-ox-LDL, ox-LDL and s-ICAM as independent variables revealed that only anti-ox-LDL and s-ICAM were significantly associated with sIL2r concentration (corrected $R^2=0.28$, $p< 0.011$). The data indicate that serum sIL2r concentration is related to plasma s-ICAM and anti-ox-LDL levels in patients with FH. These findings emphasize the role of inflammation in the development of atherosclerosis in FH patients.

Memory Stick

HYPOLIPIDEMIC EFFECTS OF *SALVIA OFFICINALIS* L. LEAVES IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

Akram Eidi[1], M. Eidi[2]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, [2]Department of Biology, Varamin Branch, Islamic Azad University, Tehran, Iran

Introduction: Sage (*Salvia officinalis* L.) has a wide range of biological activities, such as anti-oxidative properties, anti-bacterial, anti-inflammatory, fungistatic, virustatic, astringent, eupeptic and anti-hydrotic effects. Dyslipidemia, in both types 1 and 2 diabetes, plays a significant role in the manifestation and development of premature atherosclerosis leading to cardiovascular (CV) disease, and together, they are the major cause of CV morbidity and mortality in diabetic patients.

Objective: This study was designed to examine the hypolipidemic effect of sage ethanolic extract in normal and streptozotocin-induced diabetic rats.

Methods: Oral administration of sage ethanolic extract (0.1, 0.2, and 0.4 g/kg body weight) for 14 days on the level of serum triglycerides, total cholesterol, LDL and HDL in normal and streptozotocin-induced diabetic rats were evaluated.

Results: Oral administration of 0.2 and 0.4 g/kg body wt. of the sage extract for 14 days exhibited a significant reduction in serum triglycerides, total cholesterol, LDL and increased serum HDL in streptozotocin-induced diabetic rats but not in normal rats.

Conclusions: Phytochemical analysis of sage extract revealed that it contains several diterpenoids, flavonoids and glycosides. It is concluded that the traditional use of sage as a hypolipidemic agent is justified and that extracts from this plant show a dose-dependent activity. Studies are in progress to isolate and identify the active principle(s) of sage, which may be valuable in the treatment of dyslipidemia and atherosclerosis in diabetic patients.

Memory Stick

CHANGES IN PRESCRIPTION PATTERNS FOR EZETIMIBE/SIMVASTATIN, EZETIMIBE+STATIN AND STATIN THERAPIES AND EXPECTED EFFECTS ON LDL-C REDUCTION

P. Toth[1], C. Ballantyne[2,3], M. Davidson[4], D. Ramey[5], D. Neff[5], A. Tershakovec[5], H. Hu[6], J. Tomassini[5], **Kaan Tunceli**[6]

[1]Sterling Rock Falls Clinic, Sterling, IL, [2]Baylor College of Medicine, [3]Methodist DeBakey Heart and Vascular Center, Houston, TX, [4]University of Chicago, Chicago, IL, [5]Merck and Co., Inc., North Wales, PA, [6]Merck and Co., Inc., Whitehouse Station, NJ, USA

Background: Recent trends suggest decreased utilization of combination ezetimibe/simvastatin(E/S) and coadministered E+statin(E+St) therapies.

Methods: Prescription (Rx) pattern changes for E/S, E+St and St assessed using patient level data (IMS Longitudinal Rx database) during 07/14/07-1/13/08 vs 01/14/08-7/13/08 (6 mon pre/post-ENHANCE, 01/14/08). Expected LDL-C changes estimated from clinical trial data.

Results: The proportion of patients switched from E/S and E+St to other lipid-lowering therapies(LLTs) was higher 6 mons after 01/14/08 than 6 mons before (table, E/S). More patients switched to St therapy in the later period than E+St and E. Rx patterns for St were similar during both periods though fewer patients switched to E/S and E+St in the later period. Based on prior clinical data, smaller LDL-C reductions would be expected in patients who switched from E/S(~-54%) and E+St(~-56%) vs St(~-40%). Despite a switching trend toward higher St doses in the later period (eg mean doses of E/S10/33mg to S42mg), significant increases in expected LDL-C levels remained.

Conclusion: During 1/14/08-7/13/08, more patients switched from E/S and E+St to St monotherapy. LDL-C was expected to increase 10-13%. A change of this magnitude could decrease LDL-C goal attainment.

	Changes in Prescriptions for E/S (baseline therapy)**				**Expected % Change in LDL-C¶ for E/S (baseline therapy)**			
	Follow-up Therapy 07/14/07 to 01/13/08† N (%)		Follow-up Therapy 01/14/08 to 07/13/08§ N (%)		Baseline Therapy 1/14/07-7/13/07	Follow-up Therapy†† 07/14/07-01/13/08†	Baseline Therapy 7/14/07-1/13/08	Follow-up Therapy†† 01/14/08-07/13/08§
Overall	1,117,556	(100.0)	1,159,841	(100.0)	-54	-45	-54	-43
Continue	881,441	(78.9)	735,769	(63.4)	-54	-54	-54	-54
Switchers‡	67,101	(6.0)	244,294	(21.1)	-54	-37	-54	-41
Drop#	169,014	(15.1)	179,778	(15.5)	-54	0	-54	0

**Switching rates were evaluated during 12 mon periods which included 6 mon baseline therapy (most recent therapy) and 6 mon follow-up therapy phases. Patients who had ≥1 Rx for any LLT in the baseline therapy phase and those on E/S, E+St and St who had 1 Rx in either phase were included. Patients with LLT in the baseline therapy phase but no Rx of any type in the follow-up phase were excluded; Follow-up phases 6 mon before† and 6 mon after§ first reporting of ENHANCE results on 01/14/08; ‡Follow-up regimen was different than baseline therapy and included switches to St, E+St and E monotherapy; #Patients in the database who stopped use of LLT but used other Rx's; ¶Expected estimate of LDL-lowering effect of each LLT compared to no therapy based on product labels, and/or previous E trials that included various LLTs as monotherapy or coadmininstered with E; ††assumes same starting point as the baseline therapy

Memory Stick

LIPIDAEMIC PROFILE IN PATIENTS WITH ACUTE CORONARY SYNDROME IN A GREEK URBAN POPULATION

I. Kanonidis, **Konstantina Martiadou**, G. Rompolas, C. Kagiadaki, D. Eclisiarhos, G. Dadoush, G. Sakantamis

2nd Department of Cardiology, Aristotle University of Thessaloniki, Thessaloníki, Greece

Introduction: The study aims to evaluate the lipidaemic profile of patients hospitalized for acute coronary syndromes (ACS).

Material and methods: The study included all patients hospitalized for ACS during a period of 5 years in coronary care unit (CCU) of our department. On admission all patients had a complete lipid profile evaluation which included Total cholesterol, LDLC, HDLC and Triglycerides(TG), all measured in mg/dl.

Results: A total of 1426 patients were included in the study if mean age 67.06±13.29 years. Of these 998(70%) were men and 428(30%) women. 523 had an ST elevation syndrome and 833 a non ST syndrome. The mean recorded values were as follows in table.

	TChol	LDLC	HDLC	TG
ST	226.3	120.4	40.16	165.5
Non-ST	204.5	133.3	43.3	150.9

[Table 1]

Lipid disturbances were characterized as:

A) Hypercholesterolaemia in 671 patients (47%)

B) Mixed dyslipidaemia in 342 patients (24%)

C) Hypertriglyceridaemia in 612 patients (42.9%)

On the basis of recommended values for primary precaution 804 patients had normal profile (LDLC< 130), 295 patients a moderately high (130≤LDLC≤160) and 327 a high profile (LDLC>160). No significant difference was found between patients with ST and Non-ST syndromes.

Conclusions: Dyslipidaemia is a major contributing factor in 24% of patients presenting with acute coronary syndromes in Greece. No differences in lipidaemic profile was recorded in patients presented with ST or Non-ST elevation syndromes.

Memory Stick

THE METABOLIC SYNDROME CANDIDATE GENE POLYMORPHISMS AND POSTPRANDIAL CHANGES IN LDL DENSITY. THE EU LIPGENE STUDY

Aldona Dembinska-Kiec[1], M. Malczewska-Malec[1], J. Hartwich[1], D. Siedlecka[1], I. Wybranska[1], B. Kiec-Wilk[1], P. Perez-Martinez[2], C. Marin[2], J. Lopez-Miranda[2], A. Tierney[3], J. Mc Monagle[3], H. Roche[3], C. Defoort[4], P. Wolkow[5]

[1]Department of Clinical Biochemistry, Jagiellonian University Collegium Medicum, Cracow, Poland, [2]Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain, [3]University College Dublin, Dublin, Ireland, [4]INSERM U476, Marseille, France, [5]Department of Pharmacology, Jagiellonian University Collegium Medicum, Cracow, Poland

Introduction: The small dense LDL (LDL phenotype B) a component of the lipid triad associated with metabolic syndrome (MetS) and diabetes (Austin 1991). has been accepted as an cardiovascular risk factor (Berneis 2004).

Methods: The analysis was done on the subgroup of the LIPGENE Human Dietary Intervention Study (n=99). recruited MetS patients. Anthropometric characteristics, glucose tolerance parameters, plasma lipid, lipoprotein and apolipoprotein profile were measured. The association between the genotyped polymorphisms and postprandial changes in LDL density (switch from low to high, atherogenic LDL density (phenotype B)) were studied by Cochrane-Armitage trend test.

Results: LDL phenotype B correlated with plasma triglyceride (TG), but not by cholesterol (TC) level. Triglyceride Rich Lipoprotein (TRL TG, TRL C), ApoB100, ApoB48, ApoCIII and glucose were higher , when plasma ApoAI was low in the carriers of LDL phenotype B as compared to phenotype A. The SNPs significantly associated with phenotype B included the: Lipid metabolism: ABCA1, ABCG4, APOA1, APOC3, APOE, APOH, CPT1A LCAT NFATC3, OLR1, PON1, PON2, PLA2G2, PPARGC1A; Adipogenesis/inflammation: ACDC/ADIPOQ, ADIPOR, TNF; and the Insulin signalling/Glucose tolerance: CAPN10, IDE, IGF1R, INSR, SLC2A2/GLUT2, SLC2A4/GLUT4.. Among genes representing the pathway of Lipid Metabolism in LDL phenotype B carriers, the majority 14/19 (SNPs 16/23) represents higher frequency of *minor* allele.

Conclusion: The most prominent role in affecting LDL density phenotype can be assigned to genes that are controlling lipid and carbohydrate metabolism. In the prevalence of evaluated genes the minor allele was related to high LDL density.

Supported by EU FP6 FOOD-CT-2003-505944 LIPGENE project.

Memory Stick

THE EFFECT OF THE N-3/N-6 PUFA RATIO ON THE TRANSFORMATION OF POSTPRANDIAL STATE PROATHEROGENIC LDL PHENOTYPE AND OXIDATIVE STRESS PARAMETERS

Aldona Dembinska-Kiec[1], M. Malczewska -Malec[1], H. Roche[2], I. Leszczynska-Golabek[1], J. Hartwich[1], I. Wybranska[1], B. Kiec-Wilk[1], P. Perez-Martinez[3], C. Defort[4], P. Wolkow[4], J. Lopez-Miranda[3]

[1]Department of Clinical Biochemistry, Jagiellonian University Collegium Medicum, Cracow, Poland, [2]University College Dublin, Dublin, Ireland, [3]Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain, [4]Department of Pharmacology, Jagiellonian University Collegium Medicum, Cracow, Poland

Background: Postprandial lipemia oxidative stress and LDL phenotype B characteristic for metabolic syndrome (MetS) are associated with cardiovascular disease risk.The aim of the study was to evaluate the long and short term post-dietary effect of the plasma n-3/n-6 PUFA ratio on the LDL phenotype transformation, lipoprotein profile and plasma Ischemia Modified Albumin (IMA) and other oxidative stress parameters in patients with MetS.

Research design: 99 patients of the LIPGENE cohort were randomized to the one of the following isocaloric dietary regimens: Diet A High-fat (38% energy) SFA-rich diet Diet B : High-fat (38% energy), MUFA-rich diet, Diet C: low-fat (28% energy), high-complex carbohydrate diet, with 1.24g/d high oleic sunflower oil supplement., Diet D : low-fat (28% energy), high-complex carbohydrate diet supplemented with 1.24 g/d LC n-3 PUFA . Before and post-12 weeks lasting dietary intervention, patients completed an oral postprandial load test (OPLT) with the same diet composition as consumed on the assigned dietary period.

Results: All type of OPLT transiently increased plasma triglyceride followed by increased LDL density and IMA concomitantly with the decrease in LDL and HDL cholesterol. Long term administration of the diet B and D diminished plasma triglyceride and cholesterol level and the density of the main LDL subfraction. Fat modification caused by the LF n-3 PUFA resulted in favorable transformation of LDL phenotype from B to A.

Conclusion: The study demonstrates the efficacy of dietary n-3 PUFA to change the pro-atherogenic LDL phenotype to less atherogenic in patients with MetS.Supported by the FW6 EU LIPGENE.

Memory Stick

CHOLESTEROL-LOWERING EFFECT OF FORMALDEHYDE INJECTIONS IN SUPER-LOW DOSES

Elena Tereshina[1,2], S. Ivanenko[2]

[1]Russian Clinical and Scientific Center of Gerontology, Moscow, Russia, [2]World Wide Medical Assistance, Zug, Switzerland

Formaldehyde (FH) effects on tumor regression and tuberculosis recovery and protection have been registered in animals. Nonspecific homeostatic ways of FM action in the living organism were supposed. So, it was interesting to evaluate FM effect on the cholesterol homeostasis in humans. Toxicity of FM was assessed on 56 outbred ICR mice. In human studies a group of 12 volunteers with various indices of total cholesterol (TC) content (4,3 - 6,8 mmol/L) was formed. FM was injected i.m. in super low doses for five times with intervals at 7, 21, 30 and 60 days. Samples of blood were taken at 7, 28, 35 and 65 days from the beginning of the experiment. In 8 cases stable decrease in the TC content was achieved at 35 day, in 4 cases - at 7 day. In the whole group final indices of TC were 4,6-2,8 mmol/L. Within cells FM is a substrate for catalase that is located in a peroxisome and involved in the oxidation of fatty acids and synthesis of cholesterol and bile acids. At the very low values of substrate catalase reaction rate increases very rapidly. Modulating effect of FM may appear in gluthation formation and action as they both depend on CH3 group translocations. Thus, FM in super low doses may influence cholesterol homeostasis through modulation of intracellular red/ox potential.

Memory Stick

SEASONAL DIFFERENCES IN TRANS-SIALIDASES ACTIVITY

Elizaveta Orekhova[1], V. Myasoedova[1], I. Sobenin[2], A. Orekhov[1]

[1]Inst for Atherosclerosis Res, [2]Inst for General Pathology and Pathophysiology, Moscow, Russia

We have earlier found that human blood contains a soluble enzyme, belonging by its donor-acceptor properties to trans-sialidases. Incubation of low density lipoprotein (LDL) with trans-sialidase results in a loss of sialic acid from lipoprotein particles. Desialylated low density lipoprotein induces cholesterol esters accumulation, the main process accompanying

atherosclerosis development.

Lipoprotein-deficient serum obtained by ultracentrifugation was used for isolation desialylating enzyme by the affinity chromatography. Serum sample was applied on column with a-2,8-Neu5Ac-sepharose. After washing, sorbent-bound protein fraction was eluted with 50 mM sialic acid. Electrophoresis of eluted fraction under denaturating conditions revealed the presence of only one protein band with molecular weight about 67 kDa. Activity of this enzyme was measured by transfer of [H^3] labeled sialic acid from immobilized donor to soluble acceptor.

We revealed seasonal differences in trans-sialidases activity. It is well known that coronary heart disease risk decreases in summer. The majority of patients we observed had lower trans-sialidase activity in summer as compared with winter period. Thereby we could see positive correlation between seasonal fluctuation in coronary heart disease risk and activity of trans-sialidase. The highest activity was registered in December, the lowest one in July, thus desialylation in summer decreases by more than 40%.

This fact may explain seasonal variations in cardiovascular disease risk.

Memory Stick

CORRELATION BETWEEN LAG TIME OF LDL TO *IN VITRO* OXIDATION AND *IN VIVO* OX- LDL IN THE PATIENTS WITH CAD

Hashem Nayeri[1], G.A. Naderi[2], S. Asgary[2], F. Pakmehr[3], M. Sadeghi[2], E. Javadi[4]

[1]Islamic Azad University, Falavarjan Branch, Falavarjan, [2]Isfahan Cardiovascular Research Center, Isfahan University of Medical Sciences, Isfahan, [3]Food and Drug Department, Isfahan University of Medical Sciences, Iran, [4]Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: The oxidative modification of LDL is believed to play an important role in the development of atherosclerosis. Thus, measurement of plasma oxidized LDL (OX-LDL) is essential for atherosclerotic diseases, for investigating its relevance to atherosclerotic diseases. We aimed to assess the OX-LDL in patients with coronary artery disease and correlation between serum OX-LDL and in vitro susceptibility of LDL to oxidation.

Methods: Subjects of the study were selected from patients who undergone angiography (42 patients with coronary artery disease and 40 controls without any evidence of CAD).

The susceptibility of LDL to in vitro oxidation was assessed with the addition of a $CuSO_4$ solution. The lag time, propagation rate and maximal diene calculated from the oxidation curve. Biochemical factors were measured in these subjects. SPSS version15.5 was used to analyze the data, P- value under 0.05 was considered to be significant.

Results: The results indicated that the serum OX-LDL concentration was significantly elevated in CAD patients and the lag time was significantly shorter than controls ($P < 0.05$). These results clearly confirm that LDL from persons with CAD is more susceptible to oxidative modification in vitro than LDL from healthy subjects. The other measured biochemical factors were not significantly different between patients and controls ($P > 0.05$). Correlation between serum OX-LDL and susceptibility of LDL to *in vitro* oxidation did not show significant association($P > 0.05$).

Conclusion: Our findings suggest that a high OX-LDL concentration and a short LDL oxidation lag time might be independent risk factors for CAD.

Memory Stick

EVIDENCE OF INCREASED SDLDL PARTICLES DURING ACUTE INFECTION WITH EPSTEIN-BARR VIRUS

F. Apostolou[1], I. Gazi[1], V. Saougos[1], A. Tselepis[2], M. Elisaf[1], **Evangelos Liberopoulos**[1]

[1]Dept of Internal Medicine, Medical School, University of Ioannina, [2]Dept of Biochemistry, School of Chemistry, University of Ioannina, Ioannina, Greece

Lipid parameters are altered during infection and these changes may be associated with the pathogenesis of atherosclerosis. There are no data on the possible effects of acute infection (infectious mononucleosis, IM) with Epstein-Barr virus (EBV) on serum lipids and lipoproteins.

Methods: Serum lipid parameters were determined in patients with IM on diagnosis and 4 months after the resolution of infection and in age- and sex- matched controls. Fasting levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoproteins (apo) A-I, B, E and lipoprotein (a) [Lp(a)] were measured. Activities of serum paraoxonase 1 (PON1) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) as well as levels of cytokines [interleukins (IL)-1β, IL-6 and tumor necrosis factor (TNFa)] were also determined. Low-density lipoprotein (LDL) subclass analysis was performed with the Lipoprint LDL System.

Results: Twenty-nine patients (16 male, aged 24.3±14.6 years) and 20 controls were included. TC, HDL-C, LDL-C and apoA-I levels were decreased at baseline whereas apoB/apoA-I ratio and TG levels were increased compared with 4 months after the resolution of IM. At baseline, increases in inflammatory markers (IL-1β, IL-6,TNFa), in sdLDL-C levels and in Lp-PLA_2 activity were noticed while LDL particle size was decreased. The levels of lipids and lipoproteins as well as Lp-PLA_2 activity of IM patients 4 months after the resolution of the infection were comparable to the corresponding values of the control population.

Conclusions: IM is associated with atherogenic changes in serum lipids and lipoproteins. These alterations are fully restored 4 months after the resolution of the infection.

Memory Stick

LIPIDS-INDEPENDENT EFFECTS ON BRAIN ISCHEMIC STROKES

Mahnaz Talebi, V. Ehsasat, S.A. Sadredini, A. Taheragdam

Neurology, Neurosciences Research Center of Tabriz University - Medical Sciences, Tabriz, Iran

Introduction: The main mechanism leading ischemia, especially in coronary disease is atherosclerosis. But, there is little consensus that atherosclerosis is main risk factor of brain ischemia (stroke). The role of hyperlipidemia in introducing of coronary disease has been demonstrated, but not in brain vessels. The aim of this study is the assessment of lipids-independed effects on brain ischemic strokes.

Methods and materials: This study performed as case-control study in 40 patients with ischemic strokes and 40 healthy people by comparing the levels of total cholesterol, LDL, HDL, and triglycerides . The exclusion criteria was: The history of hypertension, smoking, cardioembolic strokes, diabetes, disease of the heart , hypo and hyperthyroidism, renal insufficiency, colagenvascular disease and recent use of statins or fibrates.

Results: Total cholesterol level was not differing significantly between cases and controls. HDL level was higher significantly in control than cases and HDL level less than 30mg/dl was associated with the significant increases of the risk of ischemic strokes. About LDL the results show that it's level was significantly higher in cases than controls, but despite this difference, LDL levels more than 100mg/dl was not associated with increase in risk of ischemic strokes. The level of blood triglyceride in patients was lesser than controls but the difference was not significant.

Conclusion: This study shows that low levels of serum HDL and high serum level of LDL are two main risk factors in ischemic strokes. The levels of triglyceride and total cholesterol are not associated with the risk of stroke.

Keywords: Ischemic strokes, Blood lipids, CholesteroL.

Memory Stick

A 8-WEEK AEROBIC TRAINING PROGRAM INCREASED ADIPONECTIN LEVELS IN YOUNG MALE ADULTS WITH METABOLIC SYNDROME

Miguel Angel Rosety[1], G. Fornieles-Gonzalez[2], I. Rosety[1], M. Rosety-Rodriguez[2], F.J. Ordoñez[1]

[1]School of Sport Medicine, [2]Internal Medicine, University of Cadiz, Cadiz, Spain

Background and aims: The clustering of multiple coronary artery disease risk factors in the same individual, commonly referred to as the metabolic syndrome is extremely prevalent in westernized societies. In this respect, increasing attention has been paid to plasma proteins that originate from adipose tissue, especially adiponectin, which exhibits potent anti-inflammatory and anti-atherosclerotic effects.

The present study was designed to determine the influence of regular exercise on plasmatic adiponectin levels in young male adults with metabolic syndrome.

Methods: Sixty adult men with metabolic syndrome according to the criteria reported by the National Cholesterol Education Program Adult Treatment Panel III volunteered for this study. Forty-five were randomly included in experimental group to perform an 8-week aerobic training program, 3 days/week, consisting of warm up (10-min), main part (20-35-min [increasing 5 minutes each 3 weeks]) at a work intensity of 60-75% of peak heart rate (increasing 5% each 3 weeks) and cool-down (10-min). Control group included 15 age, sex and BMI-matched men with metabolic syndrome that will not perform any program. Further our protocol was approved by an institutional ethic committee. Plasma adiponectin was assessed using a commercially available radioimmunoassay kit (HADP-61HK) 72-hours before starting the program (pre-test) and 72-hours after its ending (post-test).

Results: When compared to baseline adinopectin levels were decreased significantly after being exercised (6.5±1.1 vs 8.7±1.3 pg/ml; $p < 0.05$). On the contrary no changes were reported in controls.

Conclusions: It may be concluded regular exercise may increase plasmatic adiponectin levels in young male adults with metabolic syndrome.

Memory Stick

THE RELATIONSHIP BETWEEN LEPTIN SERUM CONCENTRATIONS AND PRODUCTION OF SMALL, DENSE LDL PARTICLES

Zeljka Vogrinc[1], M. Trbojevic-Cepe[1], J. Sertic[1], Z. Reiner[2]

[1]Department of Laboratory Diagnosis, [2]Department of Internal Medicine, Zagreb University Hospital Center, Zagreb, Croatia

Leptin, a peptide hormone secreted by adipose tissue is primarily involved in regulation of food intake and energy expenditure. Plasma leptin concentration is proportional to body adiposity and is markedly increased in obese individuals. Leptin exerts many potentially atherogenic effects such as induction of endothelial dysfunction, stimulation of inflammatory reaction, oxidative stress, proliferation of vascular smooth muscle cells, etc. Small dense low-density lipoprotein particles (sdLDL) are important risk factor for development of cardiovascular diseases. Mechanisms responsible for their formation are still unknown. We investigated the relationship between serum leptin concentrations and occurrence of sdLDL to evaluate the possible influence of this adipokine on LDL particle size distribution. Serum leptin concentrations were determined in group of 52 dyslipidemic subjects with visceral adiposity and in a group of 54 healthy individuals with normal lipid profile and normal distribution of LDL particle size. Leptin concentrations were determined by comercially available ELISA test (Mediagnost, Germany). LDL particle size determination was performed by gradient polyacrylamide gel electrophoresis developed in our laboratory. Pathologic LDL phenotype (LDL phenotype B) was detected in 27/52 dyslipidemic patients, and the mean value of leptin concentrations in this selected subgroup of patients was 14.5 mg/L. The mean value of leptin concentrations in the whole group of dyslipidemic patients was 11.4 mg/L and in the group of controls it was 8.1 mg/L. The higher levels of leptin in selected subgroup of patients with LDL phenotype B suggest that this adipokine coud be involved in mechanisms of the small, dense LDL particle production.

Memory Stick

ARYLESTERASE ACTIVITY IN NEONATES FROM THE MERIDA'S COHORT

Francisco Sanchez-Muniz[1], E. Gesteiro[2], M. Vázquez-Velasco[1], R. Lucas[1], S. Bastida[1]

[1]Nutrición, Universidad Complutense de Madrid, Madrid, [2]Nutrición Servicio de Análisis Clínicos, Hospital de Mérida, Universidad Complutense de Madrid, Mérida, Spain

Background: Paraoxonase (PON1) may be involved in lipoprotein-phospholipid metabolism and may also inhibit lipid peroxide generation in LDL. PON1 displays three main activities, paraoxonase, arylesterase, and lactonase. Up to day no data are available on arylesterase activity in newborns.

Aims: To test the arylesterase activity in neonates. To analyze the effect of sex, gestational age, anthropometrical and lipoprotein parameters on the arylesterase activity.

Material and methods: 211 Spanish singleton, normoweight, without fetal distress neonates from the Merida's cohort. Serum arylesterase activity was measured using the SBF buffer according to the Nus et al method, while anthropometry, lipids and lipoproteins measurements were obtained by using standard methods.

Results: Normality values were (mean, CI95%) arylesterase (31.0, 27.2-37.8 U/L). No significant differences between males and females at birth were observed. The arylesterase activity shows negative and significant Spearman correlations with BMI (kg/m^2) ($p=0.026$) and ponderal index (PI) (kg/m^3) ($p=0.034$) but positive with triglycerides ($p=0.044$) and the Apo A-1/Apo B ratio ($p< 0.001$).

Conclusions: The arylesterase activity found at birth was lower than that described in adults. The inverse relationships found with BMI and IP suggest a positive role of this enzyme during pregnancy. More studies are needed to assess the importance of the relationship between the arylesterase activity and the Apo A1/Apo B ratio in neonates.

This study was partially supported by the Spanish AGL 2005-07204-C0-02-01/ALI. Thanks are due to Gynecology and Obstetrician and Laboratory Services of Mérida Hospital (Badajoz, Spain) and to participant mothers and children.

Memory Stick

PORTUGUESE FAMILIAL HYPERCHOLESTEROLAEMIA STUDY: FINDING THE GENETIC DEFECT TO PREVENT PREMATURE CARDIOVASCULAR DISEASE

Ana Margarida Medeiros, A.C. Alves, S. Silva, V. Francisco, M. Bourbon, on behalf of the Investigators of the Portuguese FH Study

Dpt Promoção da Saúde e Doenças Crónicas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal

The major aim of the Portuguese FH Study is to identify the genetic cause of disease in patients with clinical diagnosis of FH to prevent the development of premature CVD.

To this date, 461 index patients with clinical diagnosis of FH and 828 affected/unaffected relatives were received to this study. The genetic diagnosis is based on the molecular study of LDLR, APOB and PCSK9 genes.

A total of 386 patients were identified genetically with FH which represent only 1,93% of the cases estimated to exist in Portugal. From the 166 index patients identified (160 with a genetic defect in *LDLR*, 4 in *APOB* and 2 in *PCSK9*), 22% of the adults had CVD and 64 individuals in these families died from premature CVD. Cascade screening of these families identified 221 individuals with FH, 12% of the adults had premature CVD. In the remaining cases where a genetic cause was not fond, 18% have premature CVD. Since these patients have a high cardiovascular risk and must have an inherited disorder, re-sequencing of *LDLR* and *APOB* by pyrosequencing methodology is being performed in order to avoid misdiagnosis of FH patients.

The genetic diagnosis of FH confirms the clinical diagnosis based on plasma cholesterol levels and provides unequivocal diagnosis of patients and early identification of relatives. The genetic diagnosis adds evidence that these patients are at high CV risk and allows for a better counselling regarding the adoption of a healthier lifestyle and treatment options, in order to reduce their risk of CHD.

Memory Stick

TO EVALUATE THE FUNCTIONS OF FOODS OR FORMULA THAT CAN REDUCE RISK PARAMETERS OF METABOLIC SYNDROME BY AN ANIMAL MODEL

Chen Chih Yu, J. Tsai

Chung Yuan University, Chung Li, Taiwan R.O.C.

Since metabolic syndrome (MS) can have three among five various parameters, it is very difficult to obtain human subjects having same syndromes for MS study. Therefore, a reliable animal model becomes important for MS studies. This study was to develop a hamster model which can evaluate the functions of a food material or formula that can reduce MS risk parameters for MS prevention. Since streptozotocin (STZ) can destroy pancreatic β-cells, STZ dose was minimized to obtain insulin resistant high blood sugar. After many tests, an AIN-93 diet modified with 22% fat, 27.95% sucrose and 20% fructose as MS inducing diet plus giving 30 mg nicotinamide (NA) + 10 mg STZ every other day showed to obtain relatively high body fat, plasma sugar, triacylglycerol, total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) or low plasma high density lipoprotein-cholesterol (HDL-C) animals. A formula (5-Mix) with five various combinations of food products, including bitter melon, licorse, green algae, red mold rice and soy protein that have been known to reduce one or more MS risk parameters were added into the MS inducing diet to confirm the animal model works well. Hamsters were given AIN-93 diet as blank (B), MS inducing diet and injected with the low dose NA + STZ as control (C) or the inducing diet with 5-Mix plus same dose NA + STZ as treatment (T) respectively for 8 weeks. Results demonstrated that this animal model is relatively suitable for MS studies.

Memory Stick

LIPID LEVELS IN 4 COHORTS OF HYPERTENSIVE PATIENTS AND THEIR RELATIONSHIP WITH BLOOD PRESSURE IN PRIMARY CARE SPAIN. TAPAS STUDY

Teresa Mantilla[1], A. Galgo[2], C. Suarez[3], A. Modrego[4], J.M. Mateo[5], B. Fernandez[6], R. Sendino[7], R. Dominguez[8], J. Gallardo[9], M. Leal[10]

[1]Primary Care Center Prosperidad, [2]Primary Care Center Espronceda, [3]Hospital Univesitario de la Princesa, Madrid, [4]Primary Care Center Buenavista, Toledo, [5]Primary Care Center El Toscar, Elche, [6]Primary Care Center Contrueces, Gijon, [7]Primary Care Center Miranda Este, Miranda de Ebro, [8]Primary Care Center San Jose Norte, Zaragoza, [9]Primary Care Center Canal de Panama, [10]AstraZeneca, Madrid, Spain

Background and aims: Clinicians treat patients whose cardiovascular risk reflects the combined effects of several risk factors that may interact. We studied the lipid levels in 4 cohorts of hypertensive patients and their relationship with blood pressure.

Methods: Retrospective study including 1678 hypertensive patients classified in 4 cohorts: cohort 1: BP not controlled in first and last visit: cohort 2: BP not controlled one year ago and good control in last visit; cohort 3: BP controlled one year ago and lost control in the last visit; cohort 4: BP controlled in both visits.

Results: Mean age was 64 years, with similar percentage male/female in each cohort. Patients in cohort 4 had lower total cholesterol (TC) than cohort 1 in both visits (204.4 vs 215.3 mg/dl p=0.0003; 201.1 vs 209.8 mg/dl p=0.0012). Change in LDL cholesterol (LDLc) followed a similar trend for cohort 4 vs cohort 1 in both visits (123.1 vs 133.2 mg/dl p=0.0002 and 120.3 vs 127.3 mg/dl p=0.0169)

Patients in all cohorts reduced TC and LDLc levels, but cohort 2 had the higher reduction. There was no difference between cohorts in high density lipoprotein cholesterol (HDLc) and triglycerides (TG).

Conclusion: Hypertensive patients with optimal BP control have lower lipid levels. Moreover, those who attain BP objectives have the higher reduction in TC and LDLc . BP control helps patients with other cardiovascular risk factor like lipids.

Memory Stick

HIGH SERUM CHOLESTEROL AND ELECTRICAL INSTABILITY IN PATIENTS WITH AN OLD MYOCARDIAL INFARCTION

Ioana Mozos, M. Hancu

Pathophysiology, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania

We hypothesized that there is a relation between serum cholesterol levels and electrical instability in patients with an old myocardial infarction. Ventricular arrhythmia and sudden cardiac death risk can be predicted using methods like: signal averaged ECG (SAECG), body surface mapping (BSM) and 12-lead ECG.

48 patients with an old myocardial infarction underwent: SAECG, BSM and 12-lead ECG.

Serum cholesterol levels were: 260±51 mg/dl, and 66.67% (32) of the patients had hypercholesterolemia (>240 mg/dl). 50% of the patients (24) had late ventricular potentials, 42% (20) a prolonged QT interval (>450 ms), and 44% (21) multipolar isointegral maps. Late ventricular potentials (LVP) were present if two of the following SAECG criteria were positive: SA-QRS (signal averaged ECG QRS duration) >120 ms, LAS40 (duration of the low-amplitude signal < 40 µV in the terminal portion of the averaged QRS complex) >38 ms, RMS40 (root mean square of the terminal 40 ms of the filtered QRS) < 20 µV. BSM QRST isointegral maps multipolarity is another noninvasive electrical instability marker.

We found a good correlation of serum cholesterol levels with the following parameters: SA-QRS (131±18 ms) (r=0.53), LAS40 (62±22 ms) (r=0.54), RMS40 (21±7 µV) (r=-0.56), isointegral QRST maxima (r=0.502), isointegral QRST minima (r=0.53), heart rate corrected QT interval: QTc (r=0.501). Serum cholesterol was significant higher in patients with a prolonged QTc (>450 ms), compared to patients with a normal QTc (280±52 mg/dl *vs.* 275±54 mg/dl) (p=0.0406).

Electrical instability is increased in patients with an old myocardial infarction and hypercholesterolemia.

Memory Stick

PLASMA PLANT STEROLS AND THE RISK OF CORONARY HEART DISEASE

Günter Fauler[1], G. Silbernagel[2], B. Winkelmann[3], B. Boehm[4], W. März[5]

[1]LB III, Clinical Institute for Medical and Chemical Laboratory Diagnosis, Graz, Austria, [2]Division of Endocrinology, Diabetology, Nephrology, Vascular Disease, and Clinical Chemistry, Department of Internal Medicine, University of Tübingen, Tübingen, [3]Cardiology Group Frankfurt-Sachsenhausen, Frankfurt, [4]Division of Endocrinology, Department of Medicine, University Hospital Ulm, Ulm, [5]Synlab Center of Laboratory Diagnostics Heidelberg, Heidelberg, Germany

Introduction: Plasma concentrations of cholesterol (C) positively correlate with atherosclerosis. Plasma C levels are determined by the balance of dietary C intake and C biosynthesis.

Objectives: Since a few years mixtures of phytosterols have been components of functional foods, because they have been demonstrated to lower cholesterol levels. Although these foods significantly can reduce plasma cholesterol levels, potential (side-) effects of phytosterols in health and/or disease are discussed.

Being controversial in their conclusions, all studies, concerning the C-lowering role of phytosterols, suggest the need of further investigations.

A contribution to this issue could be provided by the LURIC study which represents a well-defined resource for the study of environmental and genetic risk factors. Long-term follow-up on clinical events will allow to study the prognostic importance of plasma biomarkers. LURIC is a prospective cohort study of both unaffected (controls) and affected (cases) subjects at baseline.

Methods: Since many years gas chromatography-mass spectrometry (GC-MS) has been used for the determination of all kinds of sterols from different biological media. We developed a routine GC-MS method to measure plasma sterol profiles in LURIC: Quantitation data of sterols together with main plant sterols are correlated with the >2500 entries of the LURIC database.

Purpose: The purpose of this research work was to determine whether plasma plant sterol levels are associated with coronary angiographic results respectively if mildly elevated plasma plant sterol levels are associated with occurrence of coronary events.

Memory Stick

SERUM CHOLESTEROL, TRIGLYCERIDES AND THE QT INTERVAL IN HYPERTENSIVE PATIENTS

Ioana Mozos, C. Costea

Pathophysiology, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania

It was the aim of our study to evaluate a possible relation between serum cholesterol and triglycerides and the QT interval. A prolonged QT interval was previously associated with ventricular arrhythmia and sudden cardiac death risk.

35 patients with grade II essential hypertension, aged 60±12 years, underwent 12-lead ECG and Holter monitoring. Heart rate corrected QT interval (QTc) and mean QT interval in the 12 ECG leads (QTm) were assessed.

Serum cholesterol was: 242±54 mg/dl and serum triglycerides 172±47 mg/dl. 66% (23) of the patients had elevated serum cholesterol (>190 mg/dl) and 69% (24) elevated serum triglycerides (>150 mg/dl). QTc was: 430±51 ms and QTm: 386±43 ms. 31% (11) of the patients had a prolonged QTc (> 450 ms).

We found a good correlation between serum cholesterol levels and QTm (r=0.509) and between serum triglycerides and QTm (r=0.5). 5.71% of the patients with elevated serum cholesterol and triglycerides had premature ventricular contractions. The relative risk for a prolonged QTc was 5.73 in patients with hypercholesterolemia, 1.22 in patients with elevated serum triglycerides and 1.88 in hypertensive patients with both hypercholesterolemia and elevated triglycerides. The synergy index for a prolonged QTc was 0.496 when considering both elevated serum cholesterol and triglycerides.

Both elevated serum cholesterol and triglycerides are significant associated with a prolonged QTc and QTm and ventricular arrhythmia risk in hypertensive patients. Their effect is antagonistic.

Memory Stick

CORRELATION OF THE LIPIDEMIC PROFILE IN THE GENERAL POPULATION WITH AGE AND GENDER IN THE RURAL OF KASTORIA OF GREECE

Charalampos Charalampous[1], S. Patiakas[2]

[1]General Hospital of Kastoria, [2]Microbiological Laboratory, General Hospital of Kastoria, Thessaloníki, Greece

Object: To study the lipidemic profile of the general population and to scrutinize the distribution of the values depending to age and gender.

Material - method: After 12hour fasting, morning venous blood samples were taken from 605 persons (281 male and 324 female) of various ages from the general population. Using an automated biochemical analyzer, the total cholesterol (TCHOL), HDL and LDL-cholesterol and triglyceride values were determined. The results were statistically processed, depending to age and gender.

Results- conclusions: Therefore, it is proven that:

1) The average values of total cholesterol and triglycerides increase in both sexes as the age increases, with the exception of ages >65 years, where there is a decrease. Furthermore, men present increased values at a greater percentage compared to women and this difference is more intense in smaller ages.

2) The LDL average values increase while age increases.

3) The HDL average values decrease while age decreases.

Memory Stick

EFFECTS OF ALCOHOLIC EXTRACT OF *OLEA EUROPEA* L. LEAVES ON SERUM LIPIDS AND ACTIVITIES OF AMINOTRANSFERASE ENZYMES

Ramin Hajikhani, J. Solati

Biology, Islamic Azad University- Karaj Branch, Karaj, Iran

Diabetes mellitus is one of the most common endocrine metabolic disorders. we investigated antihyperlipidemic effects ethanol extracts of *Olea europea* L. leaves on serum triglycerides, cholesterol, LDL, HDL and activities of aminotransferase enzymes in streptozocin-induced diabetic adult male rats. In present study , ethanol extract of leaves of *Olea europea* L. Was found to have potent antihyperlipidemic activity that reduces blood triglycerides, level in streptozocin-induced diabetic male rats. Supplementation of this extract by gavage at dose of 0.1, 0.2 and 0.5 g/kg at 0.5ml distilled alcoholic in diabetic rats, result a significant diminution in serum triglycerides and LDL levels. Activities of serum aminotransaminase enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were changed not significantly in the extract supplemented group in respect to control group.

In conclusion, the present study demonstrates that ethanol extract of *Olea europea* L. Leaves possesses antihyperLipidemic properties thus suggesting its beneficial effect in the treatment of diabetes.

Memory Stick

HDL-CHOLESTEROL: ONE OF THE PRIME TARGETS IN THE TREATMENT OF CORONARY HEART DISEASE

Lalit M Srivastava, S. Das, A. Manocha, S. Bhargava, M. Kankra

Department of Biochemistry, Sir Ganga Ram Hospital, New Delhi, India

Epidemiological studies have proven that low serum level of HDL-Cholesterol is an independent risk factor for coronary heart disease (CHD). HDL is now emerging as target that could be rival to the approach of HMG CoA reductase inhibitors (statins) on CHD risk reduction. While there are drug therapies available which have a modest impact on serum HDL levels, our laboratory is engaged in evaluating the effect of more natural dietary components and exercise in the modulation of HDL levels. These include:

1. Ascorbic acid was fed to rabbits in different doses (15 and 25 mg /100g body weight/day) along with 0.1% cholesterol for 10 and 16 weeks. It was observed that ascorbic acid increased HDL-C levels significantly by 24 and 54% after 10 weeks and by 73 and 85 % after 16 weeks in rabbits fed with 15 and 25 mg ascorbic acid respectively. Maximum increase was observed with high doses on prolonged use.

2. Effect of moderate and high dose of alcohol were studied on HDL-C levels in male drinkers and was observed that there was a significant rise in the levels of HDL-C and the rise was especially in the HDL_2-C level.

3. A four-month programme of exercise in the form of brisk walking leads to 20-25% rise in HDL cholesterol as compared to control groups.

Further evaluation is required to ascertain whether synthetic/bioengineered drugs or food supplementation and exercise is a better option for an effective and long lasting HDL enhancement.

Memory Stick

LIPOPROTEIN(A) AND LIPID STATUS IN END STAGE RENAL DISEASE PATIENTS ON HEMODYALISIS

Danica Labudovik, K. Tosheska Trajkovska, S. Alabakovska

Department of ME Biochemistry, School of Medicine /St. Cyryl and Methodius University, Skopje, FYR Macedonia

Object: The ESRD patients on hemodyalisis have an increased risk for development atherosclerosis and CHD due to concentration of lipoprotein(a) and parameters of lipid status. The aim of this study was to determine whether the Lp(a) concentration or the concentration lipid parameters are more responsible for development atherosclerosis in ESRD patients

Methods: The plasma concentration of Lp(a), ApoA and ApoB was determined using immunonephelometrical test; total cholesterol, HDL cholesterol and triglycerides were measured using standard enzymatic test kits; LDL cholesterol was calculated by Friedewald's formula.

Results: The plasma Lp(a) concentration in ESRD patients was slightly increased but statistically insignificant compared to control group [Lp(a) 17.33 ± 17.7 mg/dl vs. 14.81±12.58mg/dl; p< 0.163). In these patients we found out dislipidemy, which was expressed as hypertriglyciredemy (1,93±0.94 vs. 1.32±0.7 mmol/L; p< 0.0005), and hypocholesterolemy (3,91±1.04 vs. 4.65±0.9mmol/L; p< 0.0004); and decreased concentration of LDL cholesterol (2,22±0.9 vs. 2.79±0.9mmol/L; p< 0.003) and HDL cholesterol (0,82±0.3 vs. 1.22±0.2 mmol/L; p< 0.0006) and ApoA1(0,98±0.21 vs. 1.19±0.19 mmol/L; p< 0.0002. The highest increase in risk carried the patients with low Lp(a) levels (< 15 mg/dl) and the highest plasma total/HDL cholesterol ratio (>5.8) (95% CI 2.10-7.58; p-4.8x10¯5)

Conclusion: we can say that the conventional risk factors are more responsible for development of atherosclerosis than plasma Lp(a) concentration in patients on hemodyalisis.

Memory Stick

POST OPERATIVE PATTERNS OF SEX HORMONES LEVEL CHANGES IN THE MALE PATIENTS UNDERGOING CABG

Tohid Emami Meybodi[1], S.K. Foruzannia[2], M.H. Abdollahi[3], S. Zare[3], H.R. Dehghan[3]

[1]Cardiovascular Research Center, Yazd University of Medical Science, [2]Department of Cardiovascular Surgery, [3]Yazd University of Medical Science, Yazd, Iran

Introduction: Sexual dysfunction is one of the most important complications that disturb the patients undergoing CABG (Coronary Artery Bypass Grafting). We evaluated the patterns the changes in sex hormones level on 40 male patients undergoing CABG.

Methods: This Cross Sectional study was evaluated the sex hormones level including; LH, FSH, prolactin, estradiol, testosterone, and DHEAS, before and 3 months after CABG on 40 male patients that operated Between December 2004 and January 2005.

Results: Mean age was 51.27± 7.8 (37 - 67) old-years. (P.V = 0.028). Estradiol was the only sex hormone that showed significant rising after CABG (P.V = 0.028)

Conclusions: We can conclude that CABG doesn't have same effect on pattern of sex hormones level changes and Estradiol is the only sex hormone that increasing after CABG and maybe it affect the sexual activity in patient after CABG

Keywords: CABG - sex hormones-complication

Memory Stick

THERE IS NO CORRELATION BETWEEN SERUM DHYDROEPIANDROSTERONE SULPHATE AND SERUM HIGH DENSITY LIPOPROTEIN LEVELS IN TURKISH POPULATION

Harun Kilic, M.G. Vural, A.G. Ertem, R. Akdemir

Diskapi Yildirim Beyazit Education and Research Hospital, Ankara, Turkey

Objective: Dehydroepiandrosterone sulphate(DHEA-S) is postulated to have antiatherogenic properties. Turkey is land of low high density lipoprotein (HDL-C). The aim of this study was to investigate the correlation between dehydroepiandrosterone and serum HDL-C levels in Turkish population.

Methods and results: Twenty patients without coronary artery artery disease

and 20 patients with coronary artery disease were examined for serum concentrations of

DHEA-S and serum lipid levels and the serum concentrations of testesteron ,

estrogen were also measured. Statistical analysis revealed no significant correlation

between serum DHEA-S level (mean DHEA-S level 181,5 ± 116mg/dl and mean HDL-C level 42,3±10,4mg/dl) and the serum concentrations HDL-C.

Conclusion: Despite unfavorable correlation between DHEA-S and serum HDL-C concentrations in our study the results of other studies indicate a potential antiatherogenic action of DHEA-S, further investigations required.

Memory Stick

CONCENTRATED ORANGE JUICE CONSUMPTION EFFECTS ON PLASMA LIPIDS, APO B AND HDL LIPID TRANSFER IN HYPERCHOLESTEROLEMIC SUBJECTS

Nancy Aptekmann[1], T. Cesar[2], M. Araújo[3], C. Vinagre[1], R. Maranhão[1]

[1]Lipid Metabolism Laboratory, Heart Institute (InCor) University of São Paulo Medical School Hospital - USP, São Paulo, [2]Food and Nutrition Department, Faculty of Pharmaceutical Sciences, [3]Food and Nutrition Department, Faculty of Pharmaceutical Sciences, Sao Paulo State University-UNESP, Araraquara, Brazil

The consumption of orange juice (**OJ**) is a habit that appears to improve the plasma lipid profile. The beneficial effects of **OJ** consumption would be particularly important if they also benefitted hypercholesterolemic subjects (**HCH**), where interventions to improve the lipid intravascular metabolism are mandatory for the prevention of atherosclerosis. To test this hypothesis, 14 **HCH** patients consumed 750mL/d of concentrated **OJ** for 60 day. Blood was sampled at the first and at the last day for plasma lipid determination and apolipoprotein profile. Lipid transfer to HDL, an important step in HDL metabolism, was evaluated by incubating plasma with a lipid donor nanoemulsion labeled with radioactive lipids. Radioactivity in the HDL fraction was then measured after chemical precipitation of apo B containing lipoproteins and the nanoemulsion. **OJ** decreased LDL-C (160±17 to 141±26mg/dL, $p< 0.01$) and apo B (128±15 to 79±32mg/dL, $p< 0.01$). However, no effect on HDL-C was observed. In regards to lipid transfers to HDL, **OJ** intake decreased the transfer to HDL of ^{3}H-phospholipids (20.6±2.4 to18.6±2.8%, $p< 0.01$), ^{3}H-triglycerides (4.9±0.5 to 3.8±1.0%, $p< 0.01$) and ^{14}C-cholesterol-ester (3.6±0.6 to 3.1±0.7%, $p< 0.01$), and increased transfer of ^{14}C-free-cholesterol (4.4±2.3 to 5.6±0.9%, $p< 0.01$). In conclusion, **OJ** intake decreased LDL-C and apo B, major risk factors for coronary artery disease. Although HDL-C and apo A1 levels were unaffected by **OJ** intake, HDL metabolism was altered. The increase in free-cholesterol transfer to HDL is probably beneficial since it facilitates the esterification of cholesterol and increases the reverse cholesterol transport.

Memory Stick

HYPOLIPIDEMIC EFFECT OF ETHANOLIC EXTRACT OF *ALLIUM AMPELOPRASUM* L. LEAVES IN HEALTHY AND DIABETIC NMARI MICE

Maryam Eidi[1], A. Eidi[2], P. Shahmohammadi[3], S. Khalaji[3]

[1]Department of Biology, Varamin Branch, Islamic Azad University, Varamin, [2]Department of Biology, Science & Research Branch, Islamic Azad University, [3]Department of Biology, Varamin Branch, Islamic Azad University, Tehran, Iran

In present study, effect of the ethanolic extract of the *Allium ampeloprasum* L. subsp. *iranicum* leaves on serum cholesterol and triglyceride levels were investigated in both normal and streptozotocin-induced diabetic NMARI mice. The animals were made diabetic using by streptozotocin (175 mg/kg, i.p.). The ethanolic extract at doses 100, 150, 200, 250, 300 and 400 mg / kg, i.p. were administered for 16 days intraperitoneally. Blood samples were obtained from heart after 16 days. The groups of normal and diabetic mice were administered saline as control groups. Serum cholesterol and triglyceride were measured by enzumatic methods. The results showed that the ethanolic extract of *Allium ampeloprasum* significantly reduced the serum cholesterol and triglyceride levels in streptozotocin-induced diabetic mice, but not in normal animals. The significant compound of leek is unknown yet. The present data indicates that extract of *Allium ampeloprasum* has hypolipidemic effect on diabetic animals. So, this plant should be considered in future therapeutic researches.

Memory Stick

HYPERCHOLESTEROLEMIC INFANT WITH A POSSIBLE LPL DEFICIENCY - A CASE REPORT

Anjali Manocha, S. Bhargava, M. Kankra, S. Das, L.M. Srivastava

Department of Biochemistry, Sir Ganga Ram Hospital, New Delhi, India

A 3-month old baby was brought to the hospital with extreme pallor and complaint of sticky stools since one month and rash on the face since one week. The patient's birth, developmental and family history were uneventful. Patient has a 7 and 5-year-old brother and sister, both asymptomatic. The significant findings on examination were pallor, xanthoma like rash on the face and a 2 cm palpable liver.

Patient's serum was thick and curdy white. Clinically, hemoglobin was estimated to be 3-4 g/dL. The only tests that could be reported were serum cholesterol 834 mg/dL and triglycerides >10,000 mg/dL. Fundoscopy revealed lipemia retinalis. The siblings were unavailable for examination and the lipid profiles of the parents did not show any abnormality. The patient was started on simvastatin.

After 1½ months of treatment the patient appeared to be improving. His hemoglobin increased to 8 mg/dL with serum cholesterol and triglycerides decreasing to 303mg/dL and 6912 mg/dL respectively. LDL was 59 and HDL 14 mg/dL.

The provisional diagnosis made was -

(i) Familial Chylomicronemia Syndrome with either Lipoprotein Lipase (LPL) Deficiency or Apolipoprotein C II deficiency or

(ii) Type V hyperlipoproteinemia

From the above clinical presentation, the probable diagnosis made was Familial Chylomicronemia / Hypertriglyceridemia possibly due to LPL deficiency. For further confirmation, estimation of LPL and Apo C-II levels would be required. Meanwhile the patient was discharged on management with simvastatin and the parents counseled. Subsequent follow up of the patient is being done to regularly monitor the clinical status.

Memory Stick

LIPID LEVEL OF PLASMA IN RATS AND THEIR BEHAVIOR AFTER SHOCK

Natalia Klyueva, S. Tsikunov, A. Denisenko, A. Pshenichnaya

Institute of Experimental Medicine RAMS, Saint Petersburg, Russia

Objectives: We studied lipid level of plasma in rats and their behavior after shock induced by threat of life.

Results: We developed a new method which allow to study shock induced by threat of life. Rats were putted up with python. We also studied posttraumatic state of rats (male and female), their behavior. We observed decreased cholesterol of high density lipoprotein and triglycerides after single shock. Decreasing of lipid level had been continued for 6 weeks. Repeating shocks were leaded to decreased total cholesterol, cholesterol of high density lipoprotein and increased triglycerides. The rats demonstrated increased immobility in Porsolt´s test-so called behavioral dispair. High aggression was obtained in intruder-resident test. Moreover, we could see patterns of pathological aggression then attack goes to attack.

Conclusions: Shock induced by threat of life of animals leads to disturbanses of behavior, that may be characterised as posttraumatis stress disorder and patologic change of lipid level.

Memory Stick

LEPTIN CONTENT IN BLOOD SERUM OF WOMEN WITH ISCHEMIC HEART DISEASE AND OBESITY

T. Suslova, O. Ogurkova, I. Kologrivova, **Olga Koshelskaya**

Institute of Cardiology, Tomsk, Russia

15 women with ischemic heart disease and obesity and 10 healthy women-volunteers were studied. Study was open-label, with control points in 3 and 6 months of treatment with Atorvastatin in 10 mg dose in group of patients. Leptin serum content (ng/ml) was detected by ELISA. LDL and HDL cholesterol levels (mmol/l) were detected by homogenous methods of direct measurement.

Obtained data revealed that women with ischemic heart disease and obesity before Atorvastatin treatment were characterized with increased content of leptin compared to the group of healthy volunteers (55.53±5.29 and 19.70±1.98 respectively, $p \leq 0.05$); 3- and 6-months therapy didn't influence on the level of hormone (54.09±4.93 and 56.73±5.60, respectively). Content of LDL cholesterol (3.35±0.13) in group of patients increased and content of HDL cholesterol (0.98±0.08) decreased compared to the group of healthy women (1.78±0.16 and 1.24±0.10 respectively), $p \leq 0.05$. During 3- and 6-months Atorvastatin therapy significant decrease of LDL cholesterol level was observed in patients (2.07±0.14 and 2.06±0.17 respectively), $p \leq 0.05$. Level of HDL cholesterol remained unchanged (0.98±0.06 and 1.06±0.11 respectively). Correlation analysis showed negative dependence between leptin and HDL cholesterol level and positive correlation between leptin and LDL levels.

Three- and six-months Atorvastatin therapy caused significant decrease of LDL cholesterol content, but didn't influence on leptin and HDL cholesterol content. Analysing obtained data one can make a conclusion that there is a distinct codependence between hyperleptinemia and atherogenic disturbances of lipid metabolism. We suppose that stable and long-term normalization of lipid metabolism and body mass might further cause the decrease of leptin level.

Memory Stick

INFLUENCE OF ATORVASTATIN ON OXIDATIVE LOW DENSITY LIPOPROTEINS MODIFICATION IN PATIENTS WITH ISCHEMIC HEART DISEASE AND ARTERIAL HYPERTENSION

O. Ogurkova, T. Suslova, I. Kologrivova, **Olga Koshelskaya**

Institute of Cardiology, Tomsk, Russia

Objective: Study the influence of six-months Atorvastatin therapy on the level of oxidative- modified low density lipoproteins (Ox-LDL), antibodies to Ox-LDL and content of C-reactive protein in patients with ischemic heart disease.

Methods: 21 patients with ischemic heart disease and arterial hypertension were studied. Study was open-label; with control point in 6 months of treatment with Atorvastatin in dose of 10 mg. Control group consisted of 14 healthy volunteers. Ox-LDL (U/l) and antibodies to Ox-LDL (mU/ml) were detected with ELISA. Level of C-reactive protein (mg/l) was detected with direct polarization fluorescence immunoassay.

Results: Obtained data revealed that increased content of Ox-LDL was observed in patients before treatment compared to the group of healthy volunteers (55.81±2.05 and 46.15±2.99 respectively, $p \leq 0.05$), as well as level of C-reactive protein (4.900±0.131 and 1.000±0.001 respectively, $p \leq 0.05$). Level of antibodies to Ox-LDL was decreased compared to healthy volunteers (414.45±88.81 and 753.89±126.37 respectively, $p \leq 0.05$). Six-months Atorvastatin treatment (therapeutic dose 10 mg) showed significant reduction of Ox-LDL (48.05±2.16) and C-reactive protein (2.650±0.088) levels in patients ($p \leq 0.05$), level of anti-Ox-LDL antibodies remained unchanged (376.27±74.37).

Conclusions:

1. In patients with ischemic heart disease increase of Ox-LDL level and C-reactive level is observed.

2. Level of antibodies to Ox-LDL is reduced in patients with ischemic heart disease compared to healthy volunteers.

3. Six-months Atorvastatin therapy in dose of 10 mg leads to the decrease of Ox-LDL and C-reactive protein content, but doesn't influence on the level of antibodies to the Ox-LDL in patients with ischemic heart disease.

Memory Stick

SERUM CHOLESTEROL, TRIGLYCERIDES AND RENAL FUNCTION IN HYPERTENSIVE PATIENTS

Ioana Mozos[1], C. Costea[1], C. Serban[1], L. Susan[2]

[1]Pathophysiology, [2]IVth Medical Clinic, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania

It has been proposed that hyperlipidemia contributes to the progression of renal disease, and, it is known that, abnormalities in lipid metabolism frequently accompany renal disease.

It was the aim of our study to find the relation between serum lipids and renal function in hypertensive patients.

69 hypertensive patients were included in the study. The following were evaluated: serum triglycerides (TGL), total cholesterol (TC), HDL and LDL cholesterol and renal function.

TGL were: 231±70 mg/dl (38% of the patients had elevated levels, > 150 mg/dl), TC: 257±45 mg/dl (90% >190 mg/dl), HDL: 32±7 mg/dl (70% < 40 mg/dl), LDL: 216±46 mg/dl (94% >115 mg/dl). Renal function was assessed using: serum uric acid (SUA): 6.7±1.51 mg/dl (9% >7 mg/dl), blood urea nitrogen (BUN): 48±10 mg/dl (62%>40 mg/dl), serum creatinine: 1.5±0.3 mg/dl (6%>1.5 mg/dl) and glomerular filtration rate (GFR): 94.8±26 ml/min/1.73 m^2 (13% < 60 ml/min/1.73 m^2 and 52%< 90 ml/min/1.73 m^2).

No significant correlation was found between serum lipids and renal function parameters (r, the Bravais-Pearson correlation coefficient between TGL and GFR was 0.21). TGL were not significant different in patients with elevated BUN (194±60 mg/dl) compared to the patients with BUN< 40 mg/dl (239±79 mg/dl) (p=0.092) and HDL also. TGL, CT, LDL and HDL were not significant different in patients with a normal or reduced GFR (< 90 ml/min/1.73 m^2).

Serum lipids are not influenced by renal function in hypertensive patients with normal, mild or moderate decreased GFR. Elevated serum lipids do not impair renal function in hypertensive patients.

Memory Stick

STRATEGIES FOR RAISING HDL AND REDUCING CHD

Ernst Schaefer

Lipid Metabolism Laboratory, Tufts University, Boston, MA, USA

There is a growing interest in raising high density lipoprotein (HDL) levels for coronary heart disease (CHD) risk reduction. Critical steps in HDL metabolism include apoA-I secretion, the uptake of cellular cholesterol via ABCA1, the esterification of free cholesterol in the presence of adequate lipolysis to form spherical HDL, and the transfer of cholesteryl ester (CE) to TRL from HDL in exchange for triglyceride, or the uptake of cholesterol from HDL by the liver. ApoA-I is then reutilized. Substantial weight loss can raise HDL cholesterol and large protective HDL, as can control of diabetes, because of more efficient lipolysis and less CE transfer to triglyceride-rich lipoproteine (TRL). Statins, especially rosuvastatin and atorvastatin, can beneficially alter HDL by enhancing TRL clearance with less CE transfer from HDL to TRL. Inhibition of CE transfer protein (CETP) is very effective in raising HDL and is currently under investigation with newer CETP inhibitors that do not raise blood pressure, aldosterone, and cortisol. These agents also delay HDL apoA-I clearance, while enhancing the clearance of apoB containing lipoproteins especially TRL. Niacin is also very effective in raising HDL and large HDL particles. We have documented that niacin not only enhances TRL apoB-100 clearance, but also increases HDL by upregulating ABCA1 and apoA-I secretion. Niacin together with a statin has been shown to be very effective in promoting regression of coronary atherosclerosis. Clinical trials are underway to determine whether adding a CETP inhibitor or niacin to a statin will lower CHD risk.

Memory Stick

FATTY ACIDS AND FATTY ALDEHYDES IN MEMBRANE PATHOLOGY OF ERYTHROCYTES IN CASE OF ANGIOPATHY STATES OF VARIOUS GENESIS

Alexander Osipenko[1], N. Kruchinsky[2]

[1]Educational Institution 'A.A. Kuleshov Mogilev State University', [2]Educational Institution, Mogilev, Belarus

The results of researches of a balance of fatty acids and fatty aldehydes of erythrocytes under conditions of hypoxia simulation and in case of vascular pathology including the coronary heart disease (CHD) and the microangiopathy against the background of the first-type pancreatic diabetes are presented. An increase in relative levels of fatty aldehydes and palmitic acid and in a level of fatty acids and aldehyde derivatives oxidized with an active form of oxygen is detected in erythrocytes of CHD patients. Changes in structure functional parameters of erythrocytes are observed. As for the patients with the first-type pancreatic diabetes, a change in the arachidonic acid /docosahexaenoic acid ratio is detected.

Memory Stick

EXPRESSIONS OF MACROPHAGE METALLOELASTASE (MMP-12) IN EXTRACELLULAR MATRIX OF ATHEROSCLEROTIC TISSUES FOLLOWING DISRUPTION OF THE VASCULAR ENDOTHELIAL SURFACE

Maimunah Mustakim[1], G.A. Froemming[2], M.S. Abdul Rahman[2], H. Nawawi[3], N. Ismail[3], E. Omar[3], M.S. Mohamed Said[4]

[1]Faculty of Health Sciences, Universiti Teknologi MARA, Bandar Puncak Alam, [2]Institute of Medical Molecular Biotechnology, [3]Faculty of Medicine, [4]Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia

Background: Susceptibility of the vascular endothelium to pro-atherogenic LDL during atherogenesis commonly affects the endothelial surface integrity. Expression of endothelial surface molecules and chemokine allows circulating monocytes to adhere and penetrate the endothelium and transform into macrophages in the vessel intima. In atherogenesis, the intimal extracellular matrix (ECM) is altered following production of matrix degrading enzymes (MMPs) by macrophages. MMP-12 is recognized to be the predominant MMPs produced in atherosclerosis as demonstrated by its increased expression during macrophage accumulation.

Objective: To correlate the expression of MMP-12 with the expression of endothelial surface molecules (VCAM, ICAM, Selectins and MCP) and ultra-structural changes of the endothelial surface during atherogenesis.

Methods: Blood serum was collected from New Zealand White Rabbits fed with 1% cholesterol for lipid profile analysis. The rabbit aorta was used to study changes in morphology and gene expressions. Morphology was examined by immunohistochemistry (IHC) and Scanning Electron Microscopy (SEM). Gene expression was analyzed by real-time PCR and QuantiGene Plex®.

Results: Lipid profiles showed highly significant difference ($p < 0.001$) of total cholesterol and LDL. IHC revealed expressions of MMP-12 in the matrix. The real-time PCR results showed significant changes in the expression of MMP-12 (36.7±1.8), means±SD comparable with data generated by QuantiGene Plex® 2.0. Endothelial surface molecules also demonstrated different expression profiles. Ultrastructural observations of the aortic luminal surface displayed endothelial defects such as globular feature and presence of numerous craters.

Conclusions: MMP-12 expression correlates with expression of endothelial dysfunction markers and changes of the ultra-structural morphology of the arterial wall in atherogenesis.

Memory Stick

ENDOTHELIAL EPHRINB2 STIMULATES DIAPEDESIS AND PRO-INFLAMMATORY ACTIVATION OF MONOCYTES AT ATHEROSCLEROSIS-PRONE SITES

Jennifer Braun, S. Hoffmann, A. Feldner, M. Hecker, T. Korff

Institute of Physiology and Pathophysiology, University of Heidelberg, Heidelberg, Germany

The arterial marker molecule ephrinB2 is known to play a pivotal role in vascular development as well as in angiogenesis. However, its function in adult arteries is poorly understood. Based on *in vitro* and *ex vivo* experiments, we have shown that ephrinB2 expression in human and mouse endothelial cells is up-regulated by cyclic stretch, an important biomechanical determinant of atherosclerosis. In line with this, immunofluorescence analyses indicated that endothelial ephrinB2 expression in wild-type mice is strongly up-regulated at atherosclerosis-prone sites of the aortic arch. By applying atomic force microscopy, we further demonstrated that human monocytes physically interact with ephrinB2 and up-regulate the ephrinB2-receptor EphB2 upon differentiation into macrophages. Likewise, adhesion of these cells to mouse aortic segments abundantly expressing ephrinB2 was inhibited by pre-treatment with soluble ephrinB2. Subsequent functional studies revealed that ephrinB2 increased the migratory activity of human monocytes and supports their transmigration through an endothelial cell monolayer. Moreover, exposure to ephrinB2 elicited a pro-inflammatory activation of these cells as evidenced by an increased expression and release of interleukin-8 and monocyte chemoattractant protein-1. In summary, these data establish ephrinB2 as an atherosclerosis-prone site-associated molecule which facilitates diapedesis and pro-inflammatory activation of monocytes and therefore may contribute to the pathogenesis of atherosclerosis.

Memory Stick

WNT5A EXPRESSION IN HUMAN ATHEROSCLEROTIC PLAQUES IS ELEVATED IN MACROPHAGE-RICH AND COMPLICATED PLAQUES

Ramiro Malgor[1], C.J. Lewis[2], C. Keller[1], L. Kohn[1], M.J. Silver[3], D.J. Goetz[4]

[1]Biomedical Sciences, [2]Biological Sciences, Ohio University, Athens, [3]MidOhio Cardiology and Vascular, Mid West Cardiology Research Foundation, Columbus, [4]Chemical and Biomolecular Engineering, Ohio University, Athens, OH, USA

Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of macrophages in the intima of large and medium sized arteries; further plaque development can ultimately trigger adverse cardiovascular events. Previously, we probed for Wnt5a protein expression in murine and human atherosclerotic lesions. Immunohistochemistry revealed the presence of Wnt5a in both murine and human plaque tissue. In the present study we sought to determine if Wnt5a expression correlated with the severity of atherosclerotic plaques. Tissue sections from the carotid arteries of patients undergoing endarterectomy were subjected to RT-PCR and immunohistochemical analysis. This analysis revealed that Wnt5a protein and mRNA expression is very low or non- detectable in regions of the artery containing less vulnerable plaques, i.e. plaques lacking active inflammation, fissures or injuries, stenosis, calcification, hemorrhaging, and significant remodeling, and/or having a thick cap and small lipid core. In contrast, plaques that were graded as complicated had detectable and significant levels of Wnt5a expression at both the RNA and protein level. Combined, these results indicate that the expression and presence of Wnt5a correlates with severity of atherosclerotic plaques and suggest that Wnt5a could be used as a biomarker for atherosclerotic disease.

Memory Stick

EXPRESSION PATTERN OF LONG PENTRAXIN 3 (PTX3) IN HUMAN ATHEROSCLEROSIS

Alexander Savchenko[1], A. Inoue[1], R. Ohashi[1], K. Inoue[2], S. Jiang[1,3], G. Hasegawa[1], M. Naito[1]

[1]Department of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, [2]Department of Cardiology, Juntendo University Nerima Hospital, [3]Perseus Proteomics Inc., Tokyo, Japan

Background and aims: Pentraxin 3 (PTX3) is the first identified long pentraxin, and it is rapidly produced and released by several cell types in response to proinflammatory signals. The aim of this study was to investigate the expression pattern of PTX3 in human atherosclerotic lesions, as well as *in vitro*.

Material and methods: We examined coronary arterial thrombi removed from patients with acute myocardial infarction. Both paraffin-embedded and frozen-fixed samples were applied for immunohistochemistry and immunofluorescence analysis using various monoclonal antibodies, including newly established monoclonal antibody against human PTX3. We also performed an immunofluorescence analysis using confocal laser microscopy, as well as immunoblotting and scanning electron microscopy on samples of polymorphonuclear neutrophils (PMNs) and six-day-cultured human monocyte-derived macrophages (Mϕs) to confirm the expression of PTX3.

Results and conclusions: We have shown that foamy Mϕs and PMNs are major cellular sources of PTX3 in the process of atherosclerotic plaque activation following rupture and thrombosis. Also, PTX3 expression depends on the stage of atherosclerosis. Of interest, PTX3 protein was found to be present following release upon stimulation with vascular chemokine IL-8 in cultured PMNs together with lactoferrin^{+}-specific granules localized in neutrophil extracellular traps (NETs) formed by extruded DNA. Moreover, extracellular expression of PTX3 protein was observed in thrombi containing abundant PMNs, suggesting the release of PTX3 protein from PMNs. Nevertheless, we did not find any evidence of PTX3 released by foamy Mϕs.

Memory Stick

THE EXPRESSION OF CYTOKINE GENES IN THE AORTA INFLUENCED BY DIET: THE EFFECT OF MCP-1 DEFICIENCY

Anna Rull, R. Beltrán-Debón, G. Aragonès, F. Rodríguez, C. Alonso-Villaverde, J. Camps, J. Joven

Centre de Recerca Biomèdica, Hospital Universitari Sant Joan de Reus, IISPV, Universitat Rovira i Virgili, Reus, Spain

Background: Monocyte chemoattractant protein-1 (MCP-1) facilitates the recruitment of monocytes/macrophages into vascular intima, and it is probably involved in the regulation of other signaling pathways relevant to the pathogenesis of arteriosclerosis and metabolic disturbances. However, chemokines are redundant. Consequently, the protective effect of MCP-1 deficiency may be mediated by changes in other cytokine signals.

Methods and results: Changes in the pattern of gene expression in the aorta were evaluated in LDLr $^{-/-}$ and MCP-1 $^{-/-}$ LDLr $^{-/-}$ mice fed either chow or Western-style diet. The effect of high-fat diet was evident. Functional analyses were used to characterize the pathways affected and to identify biological processes in which MCP-1 may play an additional role. Some data suggest that MCP-5 may act as a surrogate for MCP-1 deletion. Arteriosclerosis lesion and plaque composition are associated with enrichment in the cytokine-cytokine receptor interaction pathway.

Conclusions: There is a complex network of interactions linking MCP-1 and other cytokines. Particularly, the absence of MCP-1 limits the aortic response to atherogenic stimuli; an effect that it is even more evident when combined with a diet rich in fat and cholesterol.

Memory Stick

QUANTITATIVE PROTEOMIC APPROACH TO IDENTIFY PROTEINS INVOLVED IN ACUTE CORONARY SYNDROME

Felix Gil-Dones[1], C.M. Laborde[2], S. Alonso-Orgaz[1], J. Moreu[3], F. Vivanco[4,5], L.R. Padial[1,3], M.G. Barderas[1]

[1]Department of Vascular Pathophysiology, Hospital Nacional de Paraplejicos, [2]Biochemistry, [3]Cardiology Department, Hospital Virgen de la Salud, Toledo, [4]Inmunology Department, Fundación Jimenez Diaz, [5]Department of Biochemistry and Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain

Introduction: Acute coronary syndrome (ACS) is one of the most important cardiovascular diseases affecting developed countries worldwide. An early endothelium damage in the coronary arteries leads to an endothelial cell barrier dysfunction which allows the lipid storage and monocytes migration into the intima and means the initial stages of atherosclerosis. Thus, due to their strong association with the development of atherosclerosis, the study of plasma and circulating monocytes proteome represents one of the main pathways in the search for potential biomarkers of ACS.

Objective: Our goal was to search for differentially expressed proteins in plasma and circulating monocytes using two-dimensional difference gel electrophoresis (2D-DIGE), isobaric tag for relative and absolute quantitation (i-TRAQ) and MALDI-TOF/TOF mass spectrometry.

Material and methods: 20 patients with Non-ST-Segment Elevation ACS recruited from the cardiology service of our hospital and 20 healthy controls with up to two risk factors were used for this study. Depleted plasma samples patients and healthy controls were selected for 2D-DIGE, i-TRAQ and MALDI-TOF/TOF analyses.

Results: We found 25 differentially expressed proteins in plasma proteome using 2D-DIGE and MALDI-TOF/TOF, several of which were also identified by i-TRAQ analysis and both results were validated by inmunoblotting. Our ongoing 2D-DIGE and i-TRAQ experiments using circulating monocytes are reporting promising results as well.

Conclusions: We expect our simultaneous study of plasma and circulating monocytes proteome allows us to identify potential protein biomarkers for the future development of therapeutic strategies for ACS.

Memory Stick

ENDOPLASMIC RETICULUM STRESS INHIBITION PREVENTS THE ABCA-1 REDUCTION IN GLYCATED ALBUMIN-TREATED MACROPHAGES

G. Castilho[1], C.H. Sartori[1], A. Machado-Lima[1], E.R. Nakandakare[1], C.X.d.C. Santos[2], F.R. Laurindo[2], **Marisa Passarelli**[1]

[1]Lipids Laboratory (LIM10), Faculty of Medical Sciences of the University of Sao Paulo, [2]Vascular Biology Laboratory, Heart Institute (InCor) - University of São Paulo, Sao Paulo, Brazil

Objectives: *In vivo* and *in vitro* glycated albumin (gly-alb) reduces ABCA-1 protein levels, which is not related to alterations in ABCA-1 gene transcription or mRNA levels. We tested in macrophages the *in vitro* and *in vivo* effects of gly-alb on the endoplasmic reticulum (ER) stress and on the unfolded protein response (UPR) that trigger protein degradation.

Methods: Gly-alb was made by incubating bovine albumin with 10mM glycolaldehyde (4 days,37ºC), and control albumin (C-alb) with PBS alone. *In vivo* gly-alb (DM-alb) was isolated from uncontrolled diabetes mellitus patients serum by FPLC (HbA1c>10%). Mouse peritoneal macrophages (MPM) were incubated with C-alb or gly-alb along time and ER stress markers assessed by immunoblot. ABCA-1 content was determined by immunocytochemistry in MPM treated with gly-alb in the absence or presence of the proteasomal inhibitor (MG132, 1µM), or the ER stress inhibitor 4-phenylbutyric acid (PBA, 5mM).

Results: As compared to C-alb, gly-alb induced a time-dependent increase in Grp78,Grp94,eIf2a,ATF6 and ubiquitin indicating ER stress. No difference was observed in CHOP expression which indicates lack of apoptotic signalling. DM-alb induced greater expression of both PDI and ubiquitin comparing to albumin from non diabetics indicating cell redox imbalance and proteasomal activation, respectively. Nonetheless, PBA, but not MG132, was able to recover the ABCA-1 content in gly-alb-treated MPM.

Conclusion: Glycated albumin induces ER stress and triggers UPR adaptive pathways leading to ABCA-1 reduction in macrophages. Inhibition of the ER stress recovers the ABCA-1 content thus improving the macrophage reverse cholesterol transport in diabetes mellitus.

Funding: FAPESP (Brazil)

Memory Stick

HISTONE ACETYLATION MODIFIERS BUTYRATE AND TRICHOSTATIN A MODULATE EXPRESSION OF HISTONE DEACETYLASES (HDACS) IN VASCULAR SMOOTH MUSCLE CELLS (VSMC)

Kasturi Ranganna[1], F. Yatsu[2], O. Mathew[1]

[1]Pharmaceutical Sciences, Texas Southern University, [2]Neurology, University of Texas Health Science Center at Houston, Houston, TX, USA

Background: Besides genetic components epigenetic events are equally responsible for the etiology of many diseases including atherosclerosis and restenosis. Histone acetylation, a key epigenetic modification that controls chromatin structure and through it, gene expression is regulated by the counterbalancing activities of HDACs and histone acetyltransferases (HATs). Inhibition of HDAC activity has been shown to arrest proliferation, a key factor in atherogenesis, and induce differentiation or apoptosis by modulating gene transcription.

Aim: To assess effect of butyrate and trichostatin A (TSA) on the expression of HDACs to determine whether altered expression of HDACs also contributes to arrest of VSMC proliferation.

Methods: Cell proliferation was assessed by cell counting. Expression of HDACs and histone H3 (H3) modifications were determined by Western analysis, immunocytofluorescence, and immunoprecipitation.

Results: Butyrate and TSA treatment arrested VSMC proliferation and caused almost similar effects on the expression of HDACs and H3 modifications. However, contrary to untreated VSMC, butyrate and TSA treated VSMC exhibited downregulation of HDAC2 and HDAC5, which was confirmed by immunoprecipitation and immunocytofluorescence. Conversely, no significant difference in HDAC1 expression was observed between untreated, and butyrate and TSA treated VSMC. Moreover, acetylation of lysine9 and lysine14 of H3 was significantly increased with butyrate and TSA treatment.

Conclusions: Overall both HDAC inhibitory activity and altered expression of certain HDACs appear to contribute to induction of histone H3 acetylation by butyrate and TSA. This butyrate and TSA induced H3 acetylation appears to contribute to arrest of VSMC proliferation through the transcriptional modulation of cell cycle regulatory proteins. (NCRR/NIH/RCMI)

Memory Stick

ADIPOPHILIN LOW EXPRESSION REDUCE CELLULAR LIPID

Zhonghua Yuan, X. Liu, Q. Liu, Z. Liu, C. Tang, Z. Wang, G. Yi, L. Liu, Z. Jiang, Y. Yang

Institute of Cardiovascular Disease, Key Laboratory for Arteriosclerology of Human Province, University of South China, Hengyang, China

Objective: To explore whether adipophilin affects the expression of ACAT1 mediated by PKC signal pathway and illuminate the lipid-accumulation mechanism mediated by adipophilin.

Method: Making recombinant retroviral vector of pSuper-retro-adipophilin siRNA, transfecting it into packaging cell PA317 and getting the retrovirus. The retrovirus were infected RAW264.7 cell and got adipophilin gene low expression RAW264.7 cell line after screening with Puromycin for two weeks. The level of adipohilin and ACAT1 was determined by RT-PCR and Western Blot. Cellular lipid accumulation was determined by Oil Red O staining. The cells were incubated with atorvastatin or PKC inhibitor calphostin C for determining the mechanism.

Result: With adipophilin siRNA, RT-PCR and Western Blot showed adipophilin mRNA and protein both down-regulated significantly in RAW264.7 cells. It also made the lipid droplets decreasing, but both the blank and negative control not. As negative control, the pSuper-retro-scramble siRNA transfection could slightly down-regulated the exprssion of ACAT1, and the pSuper transfection had no effect on ACAT1 expression. But adipophilin siRNA significantly decreased ACAT1 expression. Adipophilin siRNA also declined the expression of ACAT1 after using atorvastatin which inhibited cholesterol synthesization. Adding 300nmol/L calphostin C, the expression of ACAT1 was down-regulated in RAW264.7 cells by RT-PCR and Western Blot.

Conclusion: Low expressing adipophilin down regulated ACAT1 expression and decreased the lipid accumulation in RAW264.7 cells. It suggests that Adipophilin inhibit cell lipid accumulation by ACAT1 and the PKC signal pathway.

Grants: National Natural Science Foundation (30971268), Scientific Research Foundation for Returned Overseas Chinese Scholars from Ministry of Education (2008)890.

Memory Stick

CELL PROLIFERATION IN DIFFERENT ATHEROSCLEROTIC LESIONS OF HUMAN ARTERIES

Alexander Orekhov[1,2], E. Andreeva[3], I. Adrianova[3], J. Kzhyshkowska[2], A. Gratchev[2]

[1]Institute for Atherosclerosis Research, [2]Institute of General Pathology and Pathophysiology, [3]Experimental Cardiology, Cardiology Research Center, Moscow, Russia

We studied proliferation of newly infiltrated and resident cells in atherosclerotic lesions of human aorta, carotid and coronary arteries. Investigation in grossly unaffected intima and in different types of atherosclerotic lesions (initial lesions, fatty streaks, lypofibrous plaques, fibrous plaque) revealed, that changes in the level of immune cells infiltration, the number of resident cells, and the level of cellular proliferation are similar for all studied vessels. Hematogenous cells in the lipofibrous plaques of the coronary and carotid arteries accounted for a third and almost for a half of the total cell population, respectively. In contrast, the atherosclerotic lesions of human aorta contained no more than 15% of hematogenic cells. Therefore, the contribution of hematogenous cells to the development of atherosclerosis in the carotid and the coronary artery appears to be more significant, as compared to the aorta. The differences between vessels suggest that vessel morphology and anatomic localisation contribute to the atherosclerotic lesion development. As in the aorta, in the coronary and carotid arteries a bell-shaped dependence of proliferating (PCNA+) cell number on lesion type involved in the following sequence: unaffected intima - initial lesions - fatty streaks - lypofibrous plaques - fibrous plaques was detected. The maximum number of PCNA+ resident cells was observed in lipofibrous plaques. The data obtained apparently testify to a similarity between the cellular mechanisms involved in the development of atherosclerosis in various vessels.

Memory Stick

COMPARISON OF 1.5 AND 3 TESLA MRI IN SCREENING FOR ATHEROSCLEROTIC PLAQUES IN THE ABDOMINAL AORTA IN HIGH-RISK PATIENTS

Jan-Hendrik Buhk[1], P. Bannas[1], A.-K. Finck-Wedel[1], R. Buchert[1], B. Schnackenburg[2], F.U. Beil[1], G. Adam[1], C. Weber[1]

[1]University Medical Center Hamburg Eppendorf, Hamburg, [2]Philips Medical Systems, Berlin, Germany

Objective: This prospective study compares magnetic resonance imaging (MRI) of atherosclerotic plaque of the abdominal aorta at 3 Tesla (T) versus 1.5 T in patients suffering from hereditary hyperlipidemia, a major risk factor for atherosclerosis.

Methods: MRI of the abdominal aorta at 1.5 and 3 T was performed in 21 patients (mean age 58 years). The study protocol consisted of PD-, T1-, T2- and fat saturated T2-weighted images of the abdominal aorta in corresponding orientation. Two independent radiologists performed image rating. Firstly, image quality was rated on a 5-point scale. Secondly, the atherosclerotic plaques were scored according to the modified American Heart Association (AHA) classification and analyzed for field strength-related differences. Weighted kappa statistics were calculated to assess interobserver agreement.

Results: Interobserver-agreement was substantial for nearly all categories. MRI at 3.0 Tesla offered superior image quality in all contrast weightings, most significantly in T1- and T2-weighted techniques. The majority of plaques were classified as AHA III lesions, there was no significant influence of the field strength regarding the AHA classification.

Conclusions: Abdominal aortal plaque screening is feasible at both field strengths. 3 T offers superior image quality and should therefore, if available, be preferred.

Memory Stick

***ORIGANUM MAJORANA* L. PREVENT CARDIOVASCULAR DISEASE CAUSED BY OBESITY: SUPPRESSION OF LEPTIN-INDUCED PROLIFERATION IN VASCULAR SMOOTH MUSCLE CELLS**

Ya-Mei Yu[1], Y.-W. Tzeng[2], W.-C. Chang[3]

[1]Nutrition and Health Sciences, Chang Jung Christian University, Tainan, [2]Nutrition, China Medical University, Taichung, [3]Graduate Institute of Medical Sciences, Chang Jung Christian University, Tainan, Taiwan R.O.C.

Introduction: The proliferation of vascular smooth muscle cells (VSMCs) is critical in vascular remodeling associated with atherosclerosis. Leptin, a newly discovered anti-obesity hormone, has appeared to play a pivotal role in vascular remodeling. *Origanum majorana* L. herb also called mint flower, is a traditional spice for cooking, which was produced as tea or vinegar on market in Taiwan. Ursolic acid and carnosic acid are two natural antioxidants from majorana.

Aims of study: In this study, we investigated the effect of ursolic acid and carnosic acid on the proliferation of VSMCs induced by leptin.

Results: The results indicated that both of these two compounds could lower the production of reactive oxygen species (ROS), and effectively inhibited the leptin-induced proliferation of Rat VSMCs. Ursolic acid and carnosic acid also lowered the secretion and expression of matrix metalloproteinases. (MMPs) by gelatin zymography and western blot assays, respectively. Moreover, both of them decreased the expression of ERK1/2 and nuclear translocation of nuclear factor- kappa B (NF-κB) p50, p65.

Conclusions: Our studies indicated that ursolic acid and carnosic acid may prevent the development of cardiovascular disease. Therefore, *Origanum majorana* L. plays a potential role on the prevention of cardiovascular disease in the future.

Memory Stick

IN-STENT RESTENOSIS IS ACCOMPANIED BY ELEVATION OF CIRCULATING EOSINOPHILS

Z. Gabbasov[1], S. Kozlov[2], O. Saburova[3], A. Imaeva[2], O. Vasilieva[3], S. Domogatsky[3], **Valery Kucharchuk**[2]

[1]Laboratory of Stem Cells, Institute of Experimental Cardiology, Cardiology Research Center, [2]Department of Atherosclerosis, Cardiology Research Center, [3]Institute of Experimental Cardiology, Cardiology Research Center, Moscow, Russia

Aim of investigation: The aim of this study was to assess the involvement of circulating stromal progenitor cells and blood polymorphonuclear granulocytes in the development of restenosis after sirolimus-eluting stent implantation.

Materials and methods: Follow-up angiography at 6 to 12 months was performed in 39 patients who were treated with percutaneous coronary intervention and implantation of sirolimus-eluting stents. Group 1 consisted of 15 patients who developed restenosis; group 2 consisted of 24 patients without restenosis. Levels of blood osteonectine-positive progenitor cells and polymorphonuclear granulocytes were compared in patients of these groups. Flow cytometry was used to quantitate stromal progenitor cells and eosinophils.

Results: There were no differences between the mean values of osteonectine-positive progenitor cells, neutrophils and basophils in patients with or without restenosis. The hs-CRP and IgE levels also did not differ in patients of both groups. By contrast, the mean eosinophils level was higher in patients in the group with restenosis when compared with that in the group without restenosis (262 ± 68 cells/µl versus 124 ± 67 cells/µl, $P < 0.001$). The in-stent restenosis rate was 74% in patients with eosinophils level > or = 170 cells/µl and 5% in patients with eosinophils level less than 170 cells/µl ($P < 0.001$).

Conclusions: Blood osteonectine-positive progenitor cells content did not significantly differ in patients with and without restenosis after sirolimus-eluting stent implantation. Patients with restenosis had higher level of eosinophils. It is suggested that there is a link between restenosis after sirolimus-eluting stent implantation and elevation of eosinophils in blood.

Memory Stick

EFFECTS OF COPPER AND MYELOPEROXIDASE-MODIFIED LDLS ON THE NRF2 PATHWAY IN HUMAN ENDOTHELIAL CELLS UNDER STATIC AND SHEAR STRESS CONDITIONS

Damien Calay[1], L. Caesens-Koenig[1], A. Rousseau[2], L. Mattart[1], V. Nuyens[2], N. Moguilevsky[3], K. Zouaoui Boudjeltia[2], M. Raes[1]

[1]Research Unit in Cellular Biology (URBC), University of Namur (FUNDP), Namur, [2]Experimental Medicine Laboratory, ULB, CHU Charleroi, Montigny-le-Tilleul, [3]Technology Transfer Office, University of Namur (FUNDP), Namur, Belgium

Low-density lipoprotein (LDL) oxidation is a key step in atherogenesis. Here, we compared the effects of copper-oxidized LDLs (OxLDLs) and of the more physiologically relevant myeloperoxidase-oxidized LDLs (MoxLDLs) in human Eahy926 endothelial cells, in combinaison or not with a physiological atheroprotective laminar shear stress (LSS).

Both oxidized LDLs induced an intracellular accumulation of reactive oxygen species (ROS), which was responsible for the activation of the Nrf2 transcription factor, and the subsequent overexpression of the antioxidant gene, *HO-1*. However, the intensity of these responses were different between OxLDLs and MoxLDLs. On the other hand, a physiological atheroprotective LSS also led to the nuclear translocation of Nrf2 and induced the overexpression of two Nrf2 target genes, *HO-1* and *NQO1*. Finally, the effects on the Nrf2 pathway of native, copper-oxidized, or myeloperoxidase-oxidized LDLs in combinaison with a LSS was investigated.

This study highlights that OxLDLs and MoxLDLs induce a differential activation of the Nrf2 pathway in human endothelial cells. This defensive pathway was also activated by a laminar shear stress and it will be interesting to understand the resulting combined effects of oxidized LDL and shear stress on the Nrf2 pathway.

Memory Stick

GLUCOSE, INSULIN AND LEPTIN PROMOTE MONOCYTE SURFACE EXPRESSION OF CD36 AND PHAGOCYTOSIS OF OXIDIZED-LDL THROUGH NA^+/H^+ EXCHANGER-1 ACTIVATION

M. Sarigianni[1], **Konstantinos Paletas**[1], A. Tsapas[1], M. Kaloyianni[2], C. Befani[2], G. Koliakos[3]

[1]Metabolic Diseases Unit, B Internal Medicine Clinic, Medical School, Aristotle University of Thessaloniki, [2]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, [3]Department of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Thessaloníki, Greece

Objectives: Aim of the present study was to investigate whether Na^+/H^+ exchanger-1 (NHE1) mediates the acceleration of the atherosclerotic properties of monocytes (surface expression of CD36 and phagocytosis of oxidized-LDL) by glucose, insulin and leptin in healthy lean subjects.

Methods: Monocytes were isolated from 10 lean healthy subjects. For the estimation of CD36 scavenger receptor density on monocytes surface, a fluorescein isothiocyanate (FITC)-linked monoclonal antibody was used. LDL was oxidised and labeled with DiI towards DiI-oxi-LDL and phagocytosis from monocytes was estimated through the measurement of fluorescence after 1h and 3h of incubation. All experiments were repeated after pre-incubation of monocytes with glucose or insulin or leptin alone or together with cariporide (NHE1 inhibitor).

Results: Mean age of the sample was 28.6 ±2.8. Glucose, insulin and leptin increased the surface expression of CD36 receptors ($p=0.035$, $p=0.004$, $p=0.006$, respectively). These results were counteracted after monocyte pre-incubation with cariporide ($p>0.05$ compared to control samples). Phagocytosis of oxi-LDL increased after monocyte incubation for 1h and 3h with glucose ($p=0.02$ and $p=0.032$, respectively), insulin ($p=0.015$ and $p=0.041$, respectively) or leptin ($p=0.04$ and $p=0.032$, respectively) compared to the control samples. These effects attenuated after monocyte pre-incubation with cariporide ($p< 0.025$ compared to the sample incubated with the respective hormone).

Conclusions: Atherosclerotic properties of human monocytes are accelerated by high concentrations of glucose, insulin and leptin and mediated by NHE1 activation. Inhibition of NHE1 could play a protective role against atherosclerosis.

Funding: Part of the 03ED29 research project, PENED, co-financed by National and European Community Funds.

Memory Stick

CIRCULATING MONOCYTE SUBSETS IN CRITICAL LIMB ISCHAEMIA (CLI)

Ashish Patel, A. Smith, P. Saha, J. Humphries, K. Mattock, M. Waltham, B. Modarai

Academic Department of Surgery, Cardiovascular Division, King's College London BHF Centre of Excellence, NIHR Biomedical Research Centre at Guy's & St. Thomas' NHS Foundation Trust, London, UK

Aims: Monocyte($CD14^{+ve}$) subsets expressing CD16 and Tie2 are thought to have an important role in angiogenesis and tissue remodelling. We examined the distribution of these subsets in patients with critical limb ischaemia(CLI).

Methods: Circulating mononuclear cells, expressing CD14, Tie2 and CD16, were enumerated in blood from patients with CLI, age/sex-matched and young controls, by flow cytometry. This was repeated for CLI patients 12-weeks after revascularisation/amputation. Muscle biopsies from amputated limbs were immunostained for $CD14^{+ve}$/$Tie2^{+ve}$ cells.

Results: 30 patients(19male) with CLI(mean age 70yrs±2.4sem),15 age/sex-matched(72yrs±2.5sem) and 10 young controls(30yrs±2.1sem) were recruited. Patients with CLI had over 2-fold more $CD14^{low}$/$CD16^{+ve}$($P< 0.05$) and 10-fold more $CD14^{+ve}$/$Tie2^{+ve}$ cells($P< 0.0001$) than age/sex-matched and young controls. Removal of ischaemia by revascularisation or amputation had no effect on $CD14^{low}$/$CD16^{+ve}$ cell numbers, but significantly reduced the numbers of circulating $CD14^{+ve}$/$Tie2^{+ve}$ cells($P< 0.001$).

	CLI pre-operative	**CLI post-operative**	**Controls** (Age/sex-matched)	**Controls** (Young)
$CD14^{low}CD16^{+ve}$	11.6 ± 2.2	10.7 ± 1.7	5.3 ± 1.6	4.3 ± 0.6
$CD14^{+ve}CD16^{+ve}$	39.2 ± 3.6	38.3 ± 4.5	44.0 ± 5.4	12.7 ± 3.0
$CD14^{+ve}CD16^{-ve}$	38.8 ± 4.3	41.3 ± 5.4	43.1 ± 4.7	70.5 ± 3.3
$CD14^{+ve}Tie2^{+ve}$	3.27 ± 0.34	0.56 ± 0.10	0.37 ± 0.09	0.29 ± 0.05

[Monocyte subsets in CLI]

Perivascular $CD14^{+ve}$/$Tie2^{+ve}$ cells were found in ischaemic muscle, but not normoxic muscle.

Conclusion: $CD14^{low}$/$CD16^{+ve}$ and $CD14^{+ve}$/$Tie2^{+ve}$ cells are mobilised in response to ischaemia in patients with CLI. The latter subset is known to have angiogenic properties and may represent a natural response to tissue ischaemia. A better understanding of these cells may lead to cell-based therapy that is more effective than the delivery of unselected mononuclear populations.

Memory Stick

FRACTALKINE PROMOTES A PRO-INFLAMMATORY PHENOTYPE IN HUMAN SMOOTH MUSCLE CELLS

Ana-Maria Gan[1], E. Butoi[2], D. Stan[2], M.-M. Pirvulescu[2], V. Simion[2], I. Manduteanu[2], M. Simionescu[2]

[1]Biopathology and Pharmacology of Inflammation, [2]Institute of Cellular Biology and Pathology 'Nicolae Simionescu', Bucharest, Romania

Fractalkine (Fk) is a special chemokines, which function as a cell adhesion molecule and as chemoattractant, mediating the firm adhesion and transmigration of leukocytes in the arterial wall in atherogenesis. Expression of Fk and its receptor, CX3CR1 is elevated in atherosclerotic lesions in humans and animal models, particularly at the level of smooth muscle cells (SMC). The aim of this study was to investigate the direct effects of Fk on human SMC and whether it contributes to the switch of these cells to a pro-inflammatory phenotype. To this purpose SMC in culture were exposed to 50 or 100 ng/ml fractalkine for 24 hours and then the expression of cell adhesion molecules, cytokines and chemokines was assayed. The signaling mechanisms possible involved in the process was assessed by RT-PCR, Western blot, NADPHoxidase assay and flow cytometry. Experimental and statistical data revealed that in SMC, fractalkine increased the expression of cell adhesion molecules, VCAM-1, ICAM-1, of chemokines, MCP-1, CXCL16, and of the cytokines, IL 1β, TNF, and IL6. In addition, Fk augmented the intracellular level of reactive oxygen species (ROS) and NADPHoxidase activity and generated the activation of MAPK P38 and ERK1/2 in SMC. These results indicate that Fk induces in human SMC an increased expression of pro-inflammatory molecules by a mechanism involving ROS, MAPK p38 and ERK1/2, which may be responsible (in part) to the change of the phenotype of SMC from a contractile to a pro-inflammatory type.

This work was supported by Romanian Academy and the MER-CNMP Grant No.41037.

Memory Stick

MMP-10 UP-REGULATION IN PATIENTS WITH ENHANCED THROMBIN GENERATION: EVIDENCE *IN VIVO* AND *IN VITRO* OF A THROMBIN/CD40L MEDIATED MECHANISM

S. Martínez de Lizarrondo, L. Montori, J.A. Rodríguez, C. Roncal, J.A. Páramo, J. Orbe

Atherosclerosis Laboratory, Division of Cardiovascular Sciences, Centre for Applied Medical Research (CIMA). University of Navarra, Pamplona, Spain

Objective: Thrombin activates multiple cellular types to produce inflammatory mediators such as CD40L, a member of the TNF. We analysed MMP-10 regulation by thrombin and CD40L on endothelial cells and in patients with enhanced thrombin generation.

Methods: Human umbilical vein endothelial cells (HUVEC) were stimulated with human thrombin (0.1-1 U/ml), hrCD40L (0.1-1 µg/ml) or both to assess mRNA expression (RT-PCR) and protein secretion (ELISA) for MMP-10.

Circulating CD40L, MMP-10 and D-Dimer (an indirect marker of thrombin generation) levels were measured by ELISA in 192 patients with severe sepsis at the time of diagnosis (assessed by SOFA score, lactic acid and markers of coagulopathy) and in 50 healthy controls.

Results: Thrombin and CD40L showed a synergistic effect on MMP-10 expression and secretion ($p< 0.001$). An agonist for PAR-1 (TRAP) mimicked this effect, whereas hirudin (a thrombin inhibitor), anti-PAR-1 and anti-CD40L antibodies completely neutralised MMP-10 expression.

Septic patients presented higher levels of MMP-10, CD40L and D-Dimer ($p< 0.01$) as compared to controls. MMP-10 levels correlated with lactic acid and SOFA ($p< 0.001$), whereas CD40L correlated positively with platelets ($p< 0.001$) and negatively with SOFA score ($p< 0.05$). In addition, higher circulating MMP-10 but not CD40L levels were associated with increased D-Dimer concentrations ($p< 0.01$).

Conclusion: There is strong synergism between thrombin and CD40L in vitro and in vivo that promotes marked MMP-10 up-regulation and could represent a new link between inflammation and thrombosis.

Memory Stick

A NOVEL RAT EXPERIMENTAL MODEL CONFIRMS THE CRITICAL ROLE OF METALLOPROTEINASES-2 AND 9 IN THE DEVELOPMENT OF ABDOMINAL ANEURYSMS

Karina M. Mata[1], P.S. Prudente[1], F.S. Rocha[1], C.M. Prado[1], E.M. Floriano[1], J. Elias Jr[2], E. Rizzi[3], R.F. Gerlach[4], K. Metze[5], M.A. Rossi[1], S.G. Ramos[1]

[1]Pathology, [2]Radiology, [3]Pharmacology, Faculty of Medicine of Ribeirão Preto/University of São Paulo, [4]Department of Morphology, Estomatology and Physiology, Dental School of Ribeirão Preto/University of São Paulo, Ribeirão Preto, [5]Pathology, UNICAMP, Campinas, Brazil

Introduction: Degradation of the medial elastic lamina is a hallmark of abdominal aortic aneurysms (AAAs).

Objectives: Investigate the expression of two elastolytic matrix metalloproteinases (MMPs 2 and 9) in a new rat experimental model of AAA.

Method: Male Wistar rats (150g) were randomly divided into four groups: Aneurysm (A), Injury (I), Stenosis (S) and Control (C) (40/group). The (I) group received an outside traumatic injury in the aortic wall. The (S) group received an extrinsic stenosis at a corresponding location. The (A) group received both the injury and stenosis simultaneously, and the (C) group received a sham operation. Aneurysms developed in 60-70% of the animals in group (A). Animals were euthanized at days 1, 3, 7 and 15 to accompany the induction of aneurysm. Substrate gel and *in situ* zymography were used to assess the MMPs in aorta extracts.

Results and conclusion: Animals developed AAAs by day 3 and reached a media diameter 8 times larger than normal. The aneurismal wall showed increased thickness due to damaged and fragmented elastic fibers and replacement of elastic tissue by fibrous granulation tissue. These changes were associated with an intense inflammatory infiltrate, which seemed to trigger the local production of MMPs-2 and-9, proteins that have been causally implicated in mediating widespread matrix destruction. Aortic stenosis causes turbulent flow that may alter endothelial cells homeostasis and favor the development of aneurysms. This model may help to elucidate the mechanisms triggering secretion and activation of MMPs and their contribution to the development of aneurysms.

Memory Stick

EFFECT OF QUINAPRIL ON MYOCARDIAL PERFUSION IN TYPE 2 DIABETES MELLITUS

N. Kipshidze[1], **Tamar Davarashvili**[2]

[1]Ischemic Heart Disease, National Center of Epidemiology Therapy, [2]Ischemic Heart Disease, National Center of Therapy, Tbilisi, Georgia

Background: It is estimated that micro- and macroangiopathy in T2DM results from the dysfunctions of blood vessel endothelium. Improvement of endothelial functions occupies one of the central positions in T2DM management, as it permits to reduce morbidity and mortality rates.

Aim: Study effects of Quinapril on myocardial perfusion in T2DM.

Material and methods: Have been investigated 11 normotensive patients, males (mean range 55,27±9,28 yrs) with T2DM, beside heart attack Myocardial blood circulation was studied with the use SPECT prior to and at month - 6 post treatment initiation. Two global perfusion indices were used: SSS and SRS. We studied also endothelial vasodilatation factor NO. NO was measured in venous blood prior to and at month - 6 post treatment initiation.

Results: At month-6 post treatment initiation concentration of NO increased twice. Examinations at month-6 post study initiation SPECT showed improvement of the myocardium perfusion. At rest, improvement was registered in 9 cases (81.8%); mean - by 25-30%. Stress SPECT revealed improvement in perfusion in 9 cases (81.8%) by 20%.

Discussion: We may presume that such amelioration of perfusion is related to the improvements in endothelial functions caused by the treatment with Quinapril. Correlation between the changes in the perfusion scintigraphy indices and increase in NO in these patients support the presumption (r = 0,457). Besides, all factors that, independent of Quinapril, can positively effect myocardium perfusion were excluded.

Conclusin: Thus, Quinapril improves endothelial function and consequently ameliorates myocardial perfusion in T2DM.

Keywords: Quinapril, myocardial perfusion, T2DM.

Memory Stick

HSP90 EXPRESSION AND RELEASE BY STRESSED HUMAN ENDOTHELIAL CELLS

Elisabetta Profumo[1], D. D'Arcangelo[2], A. Tagliani[3], B. Buttari[1], F. Ippoliti[3], R. Riganò[1], R. Businaro[3]

[1]Dept. of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, [2]Laboratory of Vascular Pathology, Istituto Dermopatico dell'Immacolata, [3]Sapienza University of Rome, Rome, Italy

HSP90, highly expressed in the cytoplasm of eukaryotic cells under non-stress conditions, is secreted by vascular smooth muscle cells in response to oxidative stress. In a previous study we found systemic HSP90-specific humoral and cellular immune responses in patients with carotid atherosclerosis (1) and we suggested that HSP90 may be a candidate autoantigen, target of cellular and humoral immune reactions in patients with carotid atherosclerosis (2).To verify whether oxidative stress induces HSP90 release, we treated human umbilical vein endothelial cells (HUVEC) with H_2O_2. Flow cytometry detected intracellular expression of HSP90 in 97% of untreated and 99% of H_2O_2-treated HUVEC. When incubated with H_2O_2 a small percentage of HUVEC expressed surface HSP90 (6% vs 1% untreated HUVEC). Immunoblotting disclosed soluble HSP90 in H_2O_2-treated culture supernatants. Our results indicate that in static conditions, after oxidative stress HSP90 is overexpressed and released in culture medium.We hypothesize that environmental stress leads to HSP90 hyperexpression and release in the extracellular matrix as well as in blood stream, stimulating an autoimmune response. Because this response will only target cells expressing HSP90 on their surface, it may exert a pathological role only in stressed microenvironment such as atherosclerotic plaque.

1) Riganò R. et al. Ann N Y Acad Sci 2007;1107:1-10.

2) Businaro R. et al. Atherosclerosis 2009; 207:74-83.

Memory Stick

EVALUATION OF VASCULAR REACTIVITY IN A GROUP OF PATIENTS WITH GENETIC HAEMOCHROMATOSIS

Emiddio Di Giacomo, C. Donadio, G. Carlomagno, G. Albano, A. Lucariello

Department of Internal Medicine, Cardiovascular and Immunologic Diseases, University of Naples 'Federico II', Naples, Italy

Background and aims: It has been proposed that iron would be involved in the phenomenon of atherogenesis and instability of the atherosclerotic plaque. Our work aims at correlating the presence of hyperferritinemia in patients with genetic haemochromatosis and free of traditional risk factors for atherosclerosis, with pre-clinical markers of endothelial dysfunction and atherosclerosis.

Patients and methods: 15 male patients, aged between 42 and 55 (50.7 ± 3.94), with a diagnosis of genetic haemochromatosis and free of obvious risk factors for atherosclerosis compared to 15 healthy individuals, homogeneous for sex and age and free of risk factors for atherosclerosis. All patients underwent assessment of vascular reactivity by measuring carotid intima media thickness (cIMT), a marker of pre-clinical atherosclerosis, and flow-mediated dilation (FMD), a measure of endothelium-dependent vascular reactivity and early arterial damage.

Results: Patients with genetic haemochromatosis showed significant reductions in FMD levels compared to healthy controls, (5.22 ± 1.02 vs. 14.71 ± 6.15; $p < .00001$). A significant correlation between ferritin levels and FMD has been observed ($p < .001$). Value of FMD observed in homozygous H63D individuals was significantly as compared with the other genotypes. Patients with genetic haemochromatosis showed just a little increase of the IMT values compared to healthy controls (0.91 ± 0.14 vs. 0.74 ± 0.18).

Conclusions: Endothelial function is compromised in hyperferritinemic, untreated patients with haemochromatosis and otherwise low cardiovascular risk.

Memory Stick

A CLINICAL STUDY OF AP-1 INHIBITION IN HUMAN ATHEROSCLEROSIS

Arnoud Meijer, P. le Haen, H. van Bockel, J. Lindeman

Surgery, Leiden University Medical Center, Leiden, The Netherlands

Background: Inflammation plays a key role in the initiation and progression of atherosclerotic disease. Consequently modulation of vascular inflammation is now considered a promising strategy for reducing vascular risk. The AP-1 proinflammatory pathway has been proposed as one of the key factors contributing to disease progression. We previously showed that the tetracycline analoque doxycycline attenuates aortic wall AP-1 activation in aneurysmal disease. In this study we test whether AP-1 inhibition through doxycycline improves vascular function in patients with advanced atherosclerotic disease.

Methods: We first performed a systematic analysis of AP-1 activation in the process of human atherosclerosis. Results of this analysis showed contstitutive AP-1 activation throughout all stages of atherosclerosis, and among all different plaque phenotypes. Subsequently, we tested whether quenching of AP-1 activation trough doxycycline influences vascular function in a double blind, placebo controlled cross-over study in patients with symptomatic peripheral artery disease. Vascular function was assessed by brachial flow mediated dilation and plasma samples analyzed for hs-CRP, IL-6, IL-8, ICAM-1, vWF, fibrinogen and PAI-1.

Results: Results of this evaluation showed that doxycycline slightly reduced median circulating IL-6 levels (from 1.80 to 1.61 pg/ml, p=0.05) but did not influence the other markers of vascular function and/or systemic inflammation.

Conclusion: Our studies do not characterize AP-1 as a therapeutic target for atherosclerotic disease.

Memory Stick

BASEMENT MEMBRANE ADAPTATIONS IN CRITICAL LIMB ISCHAEMIA

Robina Aslam[1], K. Khan[2], X. Shiwen[2], S. Howat[3], J. Tsui[1], D. Baker[1]

[1]Vascular Unit, Department of Surgery, [2]Department of Rheumatology, Royal Free and University College Medical School London, [3]Cardiovascular Division, King's College London, London, UK

Introduction: Muscle ischaemia is characterised by remodelling of the basement membrane (BM), which is mainly composed of type IV collagen and laminin. BM must be degraded for cell migration, and thus angiogenesis and tissue remodelling to occur. We investigated the properties skeletal muscle BM in human critical limb ischaemia (CLI).

Patients and methods: Gastrocnemious muscle samples were taken from ten CLI patients undergoing perigenicular limb amputation. Eleven control samples were obtained patients undergoing coronary artery bypass graft procedures. Microvessel BM thickness was morphometrically assessed by electron microscopy. A mean value of ten radial clockwise measurements were taken from five microvessels were per section. The expression of collagen IV and laminin (the major constituents of the BM) were examined by real-time PCR, western blotting and immunofluorescence.

Results: Morphometrically there was significant BM thickening in ischaemic muscle ($p < 0.05$). Real-time PCR showed increased gene expression of collagen IV, and a slightly reduced expression of laminin in CLI. At protein level collagen IV was significantly up-regulated in CLI ($p < 0.05$) confirming our PCR results. There was no real difference in laminin protein expression. Immunofluorescence showed localization of laminin and collagen IV to the BM. Increased collagen IV staining and decreased laminin staining in CLI was observed.

Conclusions: We have shown that CLI is associated with thickening of the BM. The main cause of the BM adaptation that we have observed is an up-regulation of collagen IV. BM thickening may limit cell migration, which would in part explain the incomplete angiogenesis that occurs in CLI.

Memory Stick

HUMAN SAPHENOUS VEIN CULTURE IN CONDITIONS OF ENDOTHELIUM DYSFUNCTION AND HYPERLIPIDEMIA: AN EX VIVO MODEL FOR EARLY DEVELOPMENT OF ATHEROSCLEROSIS

Yaowapa Maneerat[1], V. Khachansaksumeth[1], R. Nintasen[1], U. Chaisri[1], P. Viriyavejakul[1], P. Chartiburus[2]

[1]Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, [2]The Institute of Cardiovascular Diseases, Rajavithi Hospital, Bangkok, Thailand

This study aimed to develop human atherosclerosis model using tumor necrosis factor (TNF)-a and oxidized LDL (oxLDL) induced segments of normal saphenous vein culture

Materials and methods: Segments of normal human saphenous vein, obtained from patients who had bypass graft, were preincubated with TNF-alpha (5ng/ml) in complete medium (RPMI 1640 +30% fetal bovine serum) for 24 hours. Then the treated segments were cocultured with $1x10^5$ human monocytes in complete medium plus 2 mg/ml of oxLDL. After 7 days of cultivation, the segments were fixed in 1% paraformaldehyde for 24 hours at 4^oC. The morphological and functional changes compatible to critical features of early atherosclerosis were investigated by histological, histomorphometrical and immunohistochemical techniques in comparison to those in complete medium alone.

Results: Our study revealed the treated segments showed some important characters of atherosclerosis. Using standard H&E staining and Verhoeff's staining, we observed markedly thickening of tunica intima (t. intima) and t. media. The proliferation and migration of smooth muscle cell (SMC) were localized by immunostaining using antibodies to a-actin and proliferating cell nuclear antigen (PCNA). The adhesion molecules including ICAM-1 and VCAM-1 were expressed on endothelium and SMC. There were some monocytes migrated into the vascular wall and developed foam cells confirmed by special fat staining.

Discussion: Although our model possesses some critical features of atherosclerosis, long term culture are necessary for applying in pathogenesis of atherosclerosis or pharmacological studies.

Conclusion: This is the preliminary study to develop atherosclerosis model by human vessel culture

Memory Stick

DEFICIENCY OF NPP1 ATTENUATES ATHEROSCLEROTIC LESION PROGRESSION IN *APOE* KNOCKOUT MICE

Yvonne Nitschke[1], G. Weissen-Plenz[2], R. Terkeltaub[3], F. Rutsch[1]

[1]Münster University Children's Hospital, Germany, [2]Leibniz-Institute for Arteriosclerosis Research, Münster, Germany, [3]Department of Rheumatology/Immunology, VAMC, UCSD, La Jolla, CA, USA

Mutations in *ENPP1* encoding for Ecto-Nucleotide Pyrophosphatase/ Phosphodiesterase 1 (NPP1), which generates inorganic pyrophosphate, a physiologic inhibitor of hydroxyapatite deposition, are associated with medial calcification in generalized arterial calcification of infancy. The role of NPP1 in atherosclerotic lesion development is not known. *Enpp1*/*apoE* double knockout mice were generated by cross-breeding NPP1-deficient *ttw*/*ttw* mice with *apoE* null mice and were fed a high fat/ high cholesterol diet. Atherosclerotic lesion area and calcification of the aortic arch were examined. In 28 week old *Enpp1*+/-*apoE*-/- and *Enpp1*-/-*apoE*-/- mice atherosclerotic lesion size was 64% ± 20% and 59% ± 22% smaller than in *Enpp1*+/+*apoE*-/- mice (P< 0.05). However, there was no significant difference in plaque calcification, whereas only *Enpp1*-/-*apoE*-/- mice developed medial calcification. In aortic smooth muscle cells (SMCs) isolated from *Enpp1*+/-*apoE*-/- and *Enpp1*-/-*apoE*-/- mice expression of the pro-atherogenic mediator osteopontin was 14% ± 8% and 30% ± 7% less than in *Enpp1*+/+*apoE*-/- mice (P< 0.05). The present study shows that the total absence as well as the partial loss of NPP1 reduces atherogenesis in a mouse model of atherosclerosis. This effect is likely mediated by markedly decreased osteopontin expression by SMCs.

Memory Stick

MOLECULAR INVESTIGATION OF THE FUNCTIONAL RELEVANCE OF MISSENSE VARIANTS OF ICAM-1

B. Schmitz[1], P. Vischer[1], **Ralph Telgmann**[1], K. Schmidt-Petersen[1], K. Beining[1], A. Huge[1], M. Paul[2], P. Amarenco[3], F. Cambien[4], E. Brand[5], S.-M. Brand-Herrmann[1]

[1]Leibniz-Institute for Arteriosclerosis Research, University of Münster, Münster, Germany, [2]Faculty of Health, Medicine, and Life Science, Maastricht University, Maastricht, The Netherlands, [3]Department of Neurology and Stroke Centre, Bichat University Hospital, [4]INSERM, UMR S 525, Paris, France, [5]Department of Internal Medicine D, Nephrology and Hypertension, University Hospital Münster, Münster, Germany

In genome-wide studies, the ICAM-1 locus has been associated with cardiovascular and inflammatory bowel diseases. To determine the functional relevance of five missense ICAM-1 variants (G241R; I316V; P352L; K469E; R478W), we generated wild-type and variant proteins [M2(241R); M3(469E); M4(352L); M5(478W); M6(316V); M7(352L/469E)] and transiently transfected CV1 cells. RT-PCR, western blot and ELISA did not reveal any differences in mRNA and protein expression levels for any construct. Conversely, in pulse-chase experiments, compared to wild-type (90 to 120 min), M3 and M5 possessed a prolonged half-life of ~150 min, whereas M2, M4, and M7 displayed a decreased half-life of ~60 to 75 min, implying differences in protein degradation. Our results do not indicate a major impact of missense variants on ICAM-1 biological function, even if G241R and K469E were functional in pulse-chase experiments. Whether these differences in protein stability exert measurable functional consequences needs to be elucidated further.

Memory Stick

PLATELET ACTIVATION INDUCES PHENOTYPIC AND FUNCTIONAL CHANGES IN CIRCULATING MONOCYTES AS A CONSEQUENCE OF MONOCYTE-PLATELET AGGREGATE FORMATION

Gabriella Passacquale[1], V. Corrigall[2], C. Hamid[1], A. Ferro[1]

[1]Department of Clinical Pharmacology, Cardiovascular Division, [2]Department of Rheumatology, King's College London, London, UK

Objective: Circulating monocyte-platelet aggregates (MPAs) increase in response to platelet activation, and are believed to play a pathophysiological role in atherosclerosis. We investigated whether the interaction of blood monocytes with platelets may cause them to display a more pro-atherogenic phenotype.

Methods: MPAs and monocytes in peripheral blood obtained from healthy volunteers were studied by FACS analysis. Additionally, $CD14^+/CD16^-$ monocytes were isolated and co-incubated with autologous platelets. Flow cytometry and adhesion assays on pre-activated endothelial cells were performed at different time points.

Results: Three monocytic subsets were identified in blood: classical $CD14^+/CD16^-$, $CD14^{high}/CD16^+$ and $CD14^{low}/CD16^+$ cells. They displayed different expression patterns of the adhesion molecules CD11b and CD11c, although no difference was found in their ability to form MPAs *in vivo*. Platelet co-incubation enhanced monocyte phenotypic change toward $CD14^+/CD16^+$ double positivity, with CD16 expression being directly correlated to MPA levels (r^2=0.746; p=0.005). An increase in both CD11b and CD11c, together with augmented adhesion to activated endothelium, was observed in monocytes treated with platelets (11.5±1.7 vs 5.7±1.5 cells per field, when co-incubated or not with platelets respectively; p=0.005). P-selectin glycoprotein ligand-1 (PSGL-1) blocking antibody, which abrogates monocyte-platelet interaction, abolished all these effects. Moreover, co-incubation with platelets led to induction of monocyte cyclooxygenase 2 (COX-2) expression, and the COX-2 specific inhibitor NS-398 reduced platelet-dependent CD16 upregulation in $CD14^+$ monocytes, without affecting MPA formation *in vitro*.

Conclusion: Platelet interaction upregulates CD16 and expression of adhesion molecules on monocytes, in a COX-2-dependent manner, resulting in greater adhesion to the endothelium.

Memory Stick

TO DETERMINE CIRCULATING VISFATIN LEVELS IN PATIENTS WITH MYOCARDIAL INFARCTION AND ITS RELATIONSHIP WITH THE SEVERITY OF STENOSIS

Mohammad Javad Hosseinzadeh Attar[1], M. Mazaherioun[1], B. Golestan[2], A. Vasheghany Farahany[3], A. Hosseinnezhad[4]

[1]Department of Nutrition and Biochemistry, [2]Department of Biostatistics, School of Public Health, Tehran University of Medical Sciences, [3]Tehran Heart Center, [4]Endocrinology and Metabolic Research Center, Tehran University of Medical Sciences, Tehran, Iran

Objective: Visfatin recognized as a novel adiopocytokine; it has proved to possess features of proinflammatory and has been involved in the process of atherosclerosis. Due to the fact that clinical effects of visfatin on cardiovascular diseases have been neglected, the present study aimed to investigate the association of serum vistafin levels with acute myocardial infarction (AMI).

Methods: Forty-seven patients with first-ever AMI (mean age, 57.48 ±1.28 years) after 12 h of admission and 47 healthy volunteers (mean age, 56. 93±0.83years) were included in the study. The stenosis of coronary arteries was evaluated in MI patients by angiography after a week. Patients with AMI were further divided into three subgroups according to the number of significantly stenosed vessels. We examined serum visfatin through enzyme immunoassays. Biochemical parameters were analyzed. Blood pressure, body mass index (BMI), Waist circumference, smoking status, diabetes, and hypertension were recorded.

Results: Serum visfatin was significantly higher in patients with AMI compared to controls (13.06±102Vs 6.08±0.34 P=0.001). Moreover, compared to controls, patients with MI had higher diastolic and systolic blood pressure, and higher CRP. No significant correlation was found between the severity of coronary lesion and plasma visfatin levels among MI patients.

Conclusion: We detected high levels of Visfatin and CRP in patients with AMI. It is concluded that proinflammatory cytokines such as Visfatin may play an important role in the development of atherosclerosis and its complications.

Memory Stick

ENDOTHELIAL COLONY-FORMING CELL OUTGROWTH IS CORRELATED WITH INTIMA MEDIA THICKNESS AND CARDIOVASCULAR RISK FACTORS IN TYPE 2 DIABETIC PATIENTS

Harald Sourij[1], N.A. Hofmann[2], N. Tripolt[1], A. Reinisch[2], M. Eder[1], T.R. Pieber[1], D. Strunk[2]

[1]Internal Medicine, Div. of Endocrinology and Nuclear Medicine, [2]Internal Medicine, Division of Hematology, Medical University Graz, Graz, Austria

Background and aims: Endothelial colony-forming cells (ECFCs) are recently discovered circulating and vessel wall-derived endothelial progenitors with robust proliferative potential and the ability to form patent vessels in vivo. They are promising tools for future therapy in ischemic diseases. Data on ECFCs in diabetic patients are sparse. The aim of our study was to investigate the relation between cardiovascular risk factors as well as carotid atherosclerosis and ECFC outgrowth in type 2 diabetic patients.

Patients and methods: We included patients with type 2 diabetes and at least 1 additional cardiovascular risk factor. Intima media thickness was quantified by ultrasound. ECFCs were cultured directly from whole blood with a new animal protein-free humanized expansion technology.

Results: We investigated 31 patients (16m/15f; mean age 60±7 years). The total colony-outgrowth rate was 32%. Patients without colony-outgrowth had significantly higher fasting glucose levels (188±51 vs. 141±56 mg/dL, p=0.028) and significantly lower serum insulin levels (7.5±7.1 vs. 17.8±19 µU/ml, p=0.04).

Time to initial colony-formation was associated significantly with systolic blood pressure (r=0.794, p=0.01) and intima media thickness (r=0.952, p< 0.001) and inversely with HDL-cholesterol levels (r-0.803, p=0.009) in patients with colony outgrowth.

Conclusions: In type 2 diabetic patients fasting hyperglycemia and low fasting insulin levels significantly influence ECFC outgrowth. In those patients with colony outgrowth, common cardiovascular risk factors including systolic blood pressure, HDL-cholesterol and intima media thickness are associated significantly with time to initial colony formation. These data indicate a new role of ECFCs in cardiovascular physiopathology that requires further investigation.

Memory Stick

INVESTIGATION OF THE FUNCTIONAL RELEVANCE OF VCAM-1 MISSENSE VARIANTS

Boris Schmitz[1], P. Vischer[1], E. Brand[2], C. Fabritius[1], F. Cambien[3], R. Telgmann[1], S.-M. Brand-Herrmann[1]

[1]Leibniz-Institute for Arteriosclerosis Research, University of Münster, [2]Department of Internal Medicine D, Nephrology and Hypertension, University Hospital Münster, Münster, Germany, [3]INSERM, UMR S 525, Paris, France

Background and purpose: In whole genome and single gene analyses, genetic variation at the vascular cell adhesion molecule-1 (VCAM-1) locus has been associated with inflammatory disease and stroke in sickle cell anemia.

Methods and results: To determine the functional in-vitro relevance of five missense VCAM-1 variants (S318F; T384A; G413A; L555V; I716L), we generated wild-type and single variant VCAM-1-forms [MT318F, MT384A, MT413A, MT555V, MT716L] and transiently transfected CV1 cells. Semi-quantitative reverse transcription PCR, SDS-PAGE as well as immunoblot analyses did not reveal any differences with respect to mRNA and protein expression levels for any construct. The cell surface expression, measured by ELISA, did not differ across VCAM-1 forms. However, cell-cell adhesion properties varied across VCAM-1 forms; while MT318, MT384 and MT413 possessed similar adhesion properties as VCAM-1 wild-type, for MT555 and MT716 we observed a significantly reduced adhesion (~50-70% compared to wild-type; $P < 0.01$).

Conclusions: Even if the present results do not indicate a major impact of some missense variants on VCAM-1 biological function, the significantly reduced cell-cell adhesion properties of 555V and 716L compared to the remaining forms should be evaluated in further experimental approaches.

Memory Stick

INFLAMMATION MARKERS AND ENDOTHELIUM FUNCTION IN THE PATIENTS WITH ATHEROSCLEROSIS, ARTERIAL HYPERTENSION AND CHRONICAL CEREBROVASCULAR PATHOLOGY

Alla Shabalina, M.V. Kostyreva, A.A. Shabalina, M.A. Kravchenko, A.O. Chechetkin, V.G. Ionova, Y.Y. Varakin, Z.A. Suslina

Laboratory of Haemorhology and Hemostasis, Research Center of Neurology, Moscow, Russia

Purpose: To study association significance of endothelium disfunction and some inflammation markers in patients with atherosclerosis (AS), arterial hypertension (AH) and chronical cerebrovascular pathology (CCVP).

Materials and methods: 91 patients with AS and initial forms of CCVP were investigated. 50 patients made a group with mild AH (Gr.1) and 41 patients - with moderate AH (Gr.2). 24 virtually healthy persons comparable by age without AS, AH, and CCVP formed control. Endothelium function was estimated by ultrasound reactive “cuff test” (URCT). Following blood parameters reflecting endothelium function were studied: homocystein (Hcy), von Willebrand factor (vWf), cofactor Ristocetin activity of vWf (Rist-PA). TNF-α, CRP, IL-10 and fibrinogen were investigated as inflammation markers.

Results: Vasodilatation percent was showed to be validity decreased in the both groups with AH ($p< 0.05$) as compared with control. VWf was found out to be validity increased in the both groups with AH and a largest value was in Gr.2 ($p< 0.05$). Rist-PA and Hcy level were not validity varied in every group. TNF-α, fibrinogen and IL-10 were recovered to be validity raised in the both groups with AH. IL-10 was validity higher in AH-3 and TNF-α was threefold as one in control. CRP level was not differ from control.

Conclusion: Endothelium disfunction of varied intensity degree was detected in the both group. It was reflected in vasodilatation percent decreasing by URCT and raised vWf level. And TNF-α and IL-10 increasing in the patients with AS and initial forms of CCVP depended on degree of AH.

Memory Stick

ATORVASTATIN INHIBITS OXIDIZED LOW-DENSITY LIPOPROTEIN INDUCED DIFFERENTIATION OF RAW264.7 MURINE MACROPAHGES INTO DENDRITIC LIKE

Hu Liuhua, S. Linghong, B. He, S. Qing, J. Shuxuan

Shanghai Renji Hospital Affiliated to Medical College of Shanghai Jiaotong University, Shanghai, China

Dendritic cells (DCs) are professional antigen-presenting cells and have an important role in the pathogenesis of atherosclerosis. It has been confirmed that the optimal oxLDL dose (10ug/ml) can induce approximatelly 74% RAW264.7 cells differentiate into dendritic-like cells in our previous work. In this study, we examined whether atorvastatin could inhibit the differentiation of mature macrophages into DCs induced by oxLDL, since statins are lipid lowering drugs. After 24hs treatment with atorvastatin (20umol/ml), almost all the RAW264.7 cells induced by oxLDL simultaneously remained in cell size and macrophages morphology compared with those induced by oxLDL alone. Flow cytometric analysis detected reduced dendritic cell surface markers (CD40, CD86, MHC Class II and CD1d). Moreover, atorvastatin-treated RAW264.7 cells induced by oxLDL shown functional changes including increased phagocytic ability (table 1) in a time-dependent manner and reduced TNF-a as well as IL-12 p70 productin. On the whole, these data suggest dendritic-like cells origined from macrophages induced by oxLDL treatment can be inhibited by atorvastatin and this may contribute to the effect of statins on preventing the formation of atherosclerotic plaques.

	atorvastatin	oxLDL	oxLDL+atorvastatin
6h	15.89%±0.25%	18.62%±0.45% *	18.12%±0.76%
12h	23.96%±1.83%	36.50%±1.27% *	29.39%±0.50% *
24h	25.07%±0.76%	26.55%±0.37	30.10%±0.21% *

[Table 1: FITC DEXTRAN uptake (%)]

FITC DEXTRAN uptake by RAW is 17.35%±0.28%; * compared with Atorvastatin, $p < 0.05$.

Memory Stick

THE ANTI-INFLAMMATORY AND ANTYAPOPTOTIC ACTION OF HUMANIN - A NEWLY DISCOVERED 24-AMINO ACID PEPTIDE

Barbara Zapala, A. Sliwa, J. Goralska, L. Kaczynski, I. Wybranska

Clinical Biochemistry, Jagiellonian University Collegium Medicum, Cracow, Poland

Background: Humanin (HN) is a peptide which suppressed neuronal cell death caused Alzheimer's disease (AD). HN has a broad spectrum of cytoprotective, anti-inflammatory and antiapoptotic properties. Increased expression of the HN peptide observed in the AD brain cells and skeletal muscles suggests that HN is part of the physiological mechanisms promoting cell survival under stressful conditions, such as neurodegeneration, inflammation, or energy deficiency. Synthetic HN derivative Gly14-HN (HNG) has anti-inflammatory effect by lowering the levels of pro-inflammatory factors (IL-6 and TNFα) and reducing number of GFAP-immunopositive astrocytes and CD11b-immunopositive microglia.

Aims: The study of the metabolic (mitochodria-related) antiapoptotic mechanism of humanin against proinflammatory, proapoptotic, and metabolic stressors in human brain cell line, glioblastoma (LN18).

Methods: Mitochondrial metabolic potential observed by measurement of the mitochondrial oxygen production rates (OROBOROS® Oxygraph-2k) and ATP generation (Luciferase/Luciferine; ATP Lite Parkin Elmer). Global gene expression changes measured by using AFFYMETRIX microarrays (Applied Biosystems, 7900HT Fast Real-Time PCR System).

Results: Preincubation of LN18 with synthetic HN derivative Gly14-HN (4μM), treated with staurosporine (STS) (0,025 μM), a proapoptotic factor, increased the stimulated mitochondrial respiration rate and decreased ATP generation. The microarray study pointed to modification proapoptotic and proinflammatory genes of HNG revealed that BAD and CARD4 genes expression was downregualated in LN18 cells incubated with HNG and treated with STS. Genes involved in inflammatory pathway like TNFRSF25 and TNF α was down-regulated with HNG+STS.

Conclusion: HNG stimulates the mitochondrial respiration and may decrease reactive oxygen species level. Moreover HNG downregulated several genes involved in inflammatory pathway.

Memory Stick

PLASMA NITRIC OXIDE CONCENTRATIONS AND NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE

Saeedeh Salimi[1,2], M. Firoozrai[3], A. Naghavi[2], S.M. Shafiee[3], A. Mohebbi[4]

[1]Biochemistry, [2]Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, [3]Biochemistry, [4]Cardiology, Iran University of Medical Sciences, Tehran, Iran

Nitric oxide (NO) is synthesized from L-arginine by endothelium nitric oxide synthase (eNOS) encoded by the NOS3 gene on chromosome7. Since reduced NO synthesis in endothelial cells has been implicated in the development of coronary atherosclerosis; we hypothesized that polymorphisms of eNOS gene might be associated with increased susceptibility to coronary artery disease (CAD) and plasma NO concentration. We studied the endothelial nitric oxide synthase (eNOS) gene T-786C in promoter , 4a/b in intron 4 and G10T in intron23polymorphisms , in 241 unrelated CAD patients with positive coronary angiograms (>50% stenosis affected at least one coronary vessel) in Shahid Rajaii Heart Hospital and 261 age matched control subjects without a history of symptomatic CAD. The eNOS gene polymorphisms were analyzed by Polymerase Chain Reaction and RFLP. Plasma NO and lipids profile were also determined. The genotype frequencies for 4a/b in intron 4 and T-786C polymorphisms differed significantly between CAD patients and controls (P=0.041 and P=0.0001 respectively). We didn´t find the G10T polymorphism in intron23 in our population. Mean plasma NO concentration was significantly higher (P=0.0001) in CAD patients (86.7±29.4 µM) than in controls (59.7±25.1 µM). The mean plasma NOx concentrations didn't show significant differences according to genotypes of 4a/b in intron 4. In total population, but no in control group and CAD patients, the mean plasma NOx concentrations showed significant differences according to genotypes of T-786C polymorphism (p=0.004).

The logistic regression analysis revealed that smoking, NOx concentration, Family history, DBP and Cholesterol were independent risk factors of CAD.

Memory Stick

THE ROLE OF FIBRINOGEN BB-CHAIN N-TERMINUS IN PLATELET-FIBRINOGEN INTERACTIONS

Volodymyr Chernyshenko[1], T. Platonova[2]

[1]Biological Department, Kyiv National Shevchenko University, [2]Protein Structure and Function Department, Palladin Institute of Biochemistry, Kyiv, Ukraine

Binding of fibrin(ogen) to activated platelets is required for normal platelet aggregation and thrombus formation. There are platelet-binding sites on fibrinogen molecule complementary to GPIIb-IIIa platelet receptor: dodecapeptide γ(400-411) and Aα-chain RGD-sequences (Arg-Gly-Asp) - Aα(95-97), Aα(572-574).

However data about the role of N-terminal point of fibrinogen Bβ-chain in cell interactions are controversial.

We can conclude the existence of specific cell-interaction sites in above-mentioned sequences of N-terminal point of fibrinogen Bβ-chain. But these sites are not localized, their functional role is not clear and requires further investigations. Consequently we developed a model system using plasma components-free platelets and partly hydrolyzed fibrinogen desBβ(1-23). Latter was obtained using limited proteolysis of native fibrinogen by fibrinogenase from *Echis multisquamatis* venom. As we showed later, this enzyme cleaves peptide bound Bβ(Arg23-Glu24) on the initial stages of proteolysis.

The speed of ADP-induced aggregation of platelets in the presence of fibrinogen desBβ(1-23) and native protein are not statistically different. In the same time speed of collagen-induced platelets aggregation was appreciable decreased (10-15%) in the presence of fibrinogen desBβ(1-23), comparable with native fibrinogen presence. It might be supposed that this effect connected with fibrinogen interactions with platelet collagen receptor GPIIIa. It is evident that these interactions are realized (partly or completely) by the N-terminal sequences of fibrinogen β-chain.

Therefore using partly hydrolyzed fibrinogen desBβ(1-23) we defined the role of Bβ(15-23) sequences in collagen-induced platelets aggregation. The methods of limited proteolysis are useful for further localization and functional activity assay of platelet-binding sites of fibrinogen molecule.

Memory Stick

IMPACT OF CIGARETTE SMOKE ON NO BIOAVAILABILITY IN ENDOTHELIAL CELLS: ROLE OF VASCULAR NADPH OXIDASE

Yvonne Steffen

Philip Morris, Koeln, Germany

A dysfunctional endothelium is recognized as one of the earliest steps in the development of atherosclerosis. Cigarette smoking has been shown to cause endothelial dysfunction which is characterized by a reduced synthesis and/or enhanced inactivation of vasoprotective nitric oxide (NO) produced by endothelial NO synthase (eNOS). However, the mechanisms involved are not completely understood. Therefore, we investigated human umbilical vein endothelial cells (HUVECs) treated with cigarette smoke bubbled phosphate buffered saline (sbPBS) for NO levels by determining nitrite (NO_2^-) the oxidation product of NO, superoxide (O_2^-), as well as the bioavailability of NO by measuring the activity of arginase1, a co-competitor of eNOS for arginine. We found that sbPBS treatment of HUVECs resulted in (*i*) reduced NO_2-levels, (*ii*) increased levels of O_2^- and 3-nitrotyrosine, a marker of peroxynitrite generation, and (*iii*) enhanced arginase 1 activity. Treatment with the NADPH oxidase inhibitor apocynin and the xanthine oxidase inhibitor allopurinol diminished the increase of O_2^- production in HUVECs after sbPBS treatment. In experiments with isolated aortic rings apocynin and allopurinol prevented sbPBS-induced impaired vasorelaxation of rat aortas to the receptor-dependent agonist acetylcholine. Therefore we conclude that sbPBS activates NADPH oxidase and xanthine oxidase resulting in an enhanced superoxide anion production. O_2^- could react rapidly with NO to form peroxynitrite. Furthermore we speculate, that the sbPBS-induced arginase activity results in a diminished l-arginine availability to eNOS. Both mechanisms may contribute to a decreased bioavailabilty of vasoprotective NO and thereby may be involved in the cigarette smoke-dependent development of atherosclerosis.

Memory Stick

PML NUCLEAR BODY COMPONENTS PLAY A ROLE IN ADIPOCYTOKINE REGULATION

Leah Eissing[1], A. Bartelt[1], H. Sirma[2], J. Heeren[1]

[1]Institute for Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, [2]Heinrich-Pette-Institute for General Virology, Hamburg, Germany

Objective: Dysfunctional adipose tissue is an important factor in the development of diseases caused by chronic inflammation such as diabetes. The peroxisome proliferator-activated receptor gamma (PPARg) is important for adipocyte function and directly induces adiponectin expression while pro-inflammatory adipocytokine levels are modulated via transrepression. Promyelocytic leukaemia (PML) nuclear bodies are protein complexes in the nucleus of mammalian cells and have been implicated in the regulation of transcriptional processes such as the integration of cellular stress responses. However, the role of PML nuclear bodies during adipogenic differentiation as well as the integration of stress-mediated proinflammatory responses in adipocytes has not been investigated so far.

Methods: Human immortalized mesenchymal stem cells (hMSC-Tert) were stably transfected with lentiviral vectors containing shRNA against the three main PML nuclear body components PML, Daxx, and Sp100, respectively. Adipocyte differentiation (Prawitt et al., 2008) as well as adipocytokine expression before and after stimulation with TNFa or rosiglitazone were analysed by quantitative real time PCR and ELISA.

Results: The lack of the PML body components did not significantly influence adipocyte differentiation. However, adipocytokine expression levels were strongly affected in the knockdown cell lines: while PML, Daxx, and Sp100 have stimulatory effects on IL6 expression and secretion, adiponectin levels are strongly repressed.

Conclusion: The PML nuclear body components PML,Daxx and Sp100 play a prominent role in the complex regulation of adipocytokine expression. This effect can be explained by the direct interaction of lipid-activated PPARg with PML nuclear body proteins.

Memory Stick

DECREASED EXPRESSION OF CD39 IN SYMPTOMATIC CAROTID ARTERY PLAQUES

Yuichiro Sato, S. Moriguchi-Goto, K. Hatakeyama, K. Marutsuka, Y. Asada

University of Miyazaki, Miyazaki, Japan

Background: Thrombus formation complicated by carotid plaque disruption plays a critical role in the pathogenesis of stroke. CD39 is an extracellular enzyme of ATP and ADP and terminates platelet aggregation response to ADP. However, CD39 expression in carotid artery plaque and its relation to thrombus formation have not been investigated.

Methods: Atherosclerotic carotid plaques were obtained from 35 patients (symptomatic=20, asymptomatic=15) undergoing carotid endoarterectomy. We compared the frequency of thrombosis, plaque disruption, the plaque components (macrophages, smooth muscle cells, red blood cells), and expression of CD39 were assessed histologically between symptomatic and asymptomatic plaques.

Results: The higher incidence of thrombus formation was observed in plaques with symptoms (55%) than in those without symptoms (13%) ($P< 0.05$). The incidence of plaque disruption was higher in plaques with symtoms than in those without symptoms, but not significant. In addition, symptomatic plaques were characterized decreasing of smooth muscle cells, but there was significantly difference between groups in the percentage of macrophages, or red blood cells. CD39 was mainly expressed in smooth muscle cells. CD39 immunoreactivity of smooth muscle cells was significantly decreased in symptomatic plaques compared with those of asymptomatic plaques ($P< 0.05$).

Conclusion: These results suggest that decreasing smooth muscle cells and CD39 depression in carotid plaque plays an important role in thrombus formation.

Memory Stick

IMPACT OF ANDROGENS ON VASCULAR CELL PHYSIOLOGY

Shanhong Ling, L. Nazareth, L. Nheu, P. Komesaroff, Hormones and the Vasculature Group

Medicine, Monash University Central Clinical School, Melbourne, VIC, Australia

Atherosclerosis predominantly occurs in males, with strong correlations of male hormones androgens. This study aims to examine the impact of androgens on the physiology of vascular cells. Vascular endothelial (EC) and smooth muscle cells (VSMC) in cultures were incubated with androgen testosterone (T), dihydrotestosterone (DHT) or dehydroepiandrostenedione (DHEA) at physiological (5nM) and supraphysiological (50nM) concentrations. Cell growth and death, DNA and collagen synthesis, and gene protein expression were assessed. The study showed that (1) DHEA protected EC from superoxide injury via androgen receptor (AR)-independent mechanism; (2) T induced DNA synthesis and cell growth in EC with an activation of ERK1/2 activity, which was not blocked by AR antagonist Flutamide (100nM), indicating an AR-independent action; (3) DHT inhibited DNA synthesis and cell growth in EC via AR-dependent manner; (4) T stimulated VSMC proliferation, which was not blocked by Flutamide; and (5) T and DHT reduced collagen synthesis in VSMC via AR-dependent mechanism. Thus, androgens produce multiple effects on vascular cells via either AR-dependent or -independent mechanisms. Some effects, such as protection of EC injury by DHEA, enhancement of EC growth by T, and inhibition of collagen synthesis by T and DHT, may be “beneficial” in preventing atherosclerosis. In contrast, the effects, such as reduction of EC growth by DHT and stimulation of VSMC proliferation by T, may be “harmful” on blood vessels in atherogenesis. Further study needs to assess the significance of these *in vitro* effects of androgens in the vascular function and development of atherosclerosis under *in vivo* conditions.

Memory Stick

KLF2 INDUCES SPECIFIC ACTIN SHEAR FIBRES ESSENTIAL FOR FLOW-ALIGNMENT AND PHENOTYPE OF VASCULAR ENDOTHELIUM

Thomas A Leyen[1], R.A. Boon[1], R.D. Fontijn[1], G.P. van Nieuw Amerongen[2], A.J.G. Horrevoets[1]

[1]Molecular Cell Bioloy and Immunology, [2]Physiology, VU University Medical Center, Amsterdam, The Netherlands

Introduction: Absence of shear stress at arterial bends and bifurcations induces atherosclerotic lesions. Krüppel-like factor 2 (KLF2) is a major effector of the beneficial effects of shear stress on endothelial cells by affecting proteins related the cytoskeleton. We set out to explore the implications of this on anti-inflammatory effects of endothelium.

Methods: Human HUVEC, microvascular, and arterial endothelium grown on fibronectin-coated coverslips were transduced with KLF2-expressing or anti-KLF2 silencer RNA lentiviral vectors and exposed to arterial levels of flow in IBIDI laminar flow chambers. The cells were fluorescently stained for focal adhesion proteins and analyzed by 3-colour Confocal scanning or Deconvoluting microscopy. Effects on signalling and transcriptome were determined by quantitative Western blotting and real-time PCR.

Results: Both flow and KLF2-induced specific actin shear fibres are thick cables connected at both ends to focal adhesions running across the basal membrane of the cells. The coinciding changes in focal adhesion complexes regulate actin-structure modulating proteins. KLF2-induced fibers are essential for flow alignment and contain force-generating phosphomyosin. Fibers were formed independent from Rho kinase and quite distinct from conventional stress fibers, but similar to fibers in ex vivo arteries and veins. The presence of actin shear fibres affects the expression of a panel of proinflammatory genes, by suppressing signaling through c-jun N-terminal kinase (JNK) and its target pro-inflammatory transcription factor ATF2

Conclusions: KLF2 directs actin architecture through regulation of focal adhesion activity in various endothelial cells, which is essential for its anti-inflammatory effects on endothelium.

Memory Stick

UROKINASE PLASMINOGEN ACTIVATOR (UPA) PROVOKES MACROPHAGE ATHEROGENICITY AND APOPTOSIS

Bianca Fuhrman, J. Khateeb, A. Gantman, M. Aviram

The Lipid Research Laboratory, Technion Faculty of Medicine and Rambam Medical Center, Haifa, Israel

Background and aims: Macrophages in human atherosclerotic lesions express urokinase plasminogen activator (uPA), and its receptor, uPAR. The uPA/uPAR system has been shown to contribute to atherosclerosis progression and to be involved in the weakening of the atherosclerotic plaque. The atherosclerotic plaque is dominated by macrophage-foam cells loaded with cholesterol and oxidized lipids. Our goal is to study the functions of the uPA/uPAR system in atherosclerosis, in relation to macrophage-foam cell formation.

Methods and results: uPA significantly increased macrophage unesterified cholesterol content, resulting from an increase in cellular cholesterol biosynthesis. This effect required the binding of uPA to its receptor, uPAR, and was mediated via activation of SREBP-1 through PI3K and MEK signal transduction pathways. Furthermore, uPA increased macrophage oxidative stress by enhancing NADPH oxidase activation. In turn, uPA initiated an oxidative stress-response by enhancing the expression of macrophage paraoxonase 2 (PON2) through the PI3K-NADPH oxidase-reactive oxygen species (ROS)-MEK-SREBP2 signaling cascade activation. Platelet-derived growth factor receptor b (PDGFR-beta) served as an accessory transmembrane adaptor molecule, which in response to uPA binding to uPAR, mediated the activation of intracellular signal transduction. Moreover, uPA induced macrophage apoptosis, as measured by the loss of mitochondrial membrane potential or by annexinV/PI staining.

Conclusions: The ability of uPA to regulate cholesterol accumulation, oxidative stress and apoptosis in macrophages represents a new paradigm by which uPA is implicated in atherogenic processes, beyond its role in the fibrinolytic system. These findings provide new insight into the relationship between uPA and vulnerable plaque.

Memory Stick

RESISTIN AND HIGH GLUCOSE MODULATE THE EXPRESSION OF FRACTALKINE RECEPTOR (CX3CR1) IN HUMAN MONOCYTES

Daniela Stan[1], M. Calin[2], E. Butoi[2], A.-M. Gan[2], M.M. Pirvulescu[2], V. Simion[2], I. Manduteanu[2], M. Simionescu[2]

[1]Biopathology and Pharmacology of Inflammation, [2]Institute of Cellular Biology and Pathology 'Nicolae Simionescu', Bucharest, Romania

Fractalkine (Fk), a chemokine with a dual function, cell adhesion molecule and chemoatractant has an important role in inflammatory diseases such as atherosclerosis. Fractalkine's action is mediated by its specific receptor CX3CR1. Resistin is a cytokine with an unclear function in humans where it is assumed to be involved in inflammation. In this study we searched for the effect of resistin and high glucose concentration (HG) on the expression of CX3CR1 in human monocytes. In addition we evaluated CX3CR1 expression in peripheral blood mononuclear cells (PBMC) isolated from patients with different inflammatory diseases. Monocytes (U937 cells) were incubated with HG (25mM) and/or resistin (100ng/ml) for 24h at 37ºC, and the gene and protein expression of CX3CR1 were determined by RT-PCR and Western-Blot and flow-cytometry, respectively. The results showed that HG and resistin increased the gene and protein expression of monocytes CX3CR1. Co-stimulation with resistin and HG induced CX3CR1 expression at similar levels as every inducer alone. The expression of the CX3CR1 in PBMC isolated from patients with rheumatoid arthritis was higher as compared with the patients with hyperglycemia and hypertension. Furthermore, CX3CR1 expression was increased in PBMC isolated from patients with metabolic syndrome in comparison with patients with hyperglycemia alone. In conclusion resistin and HG modulate the expression of CX3CR1 receptor in monocytes; in PBMC isolated from patients with different inflammatory diseases, the receptor expression is also increased but the level varies according to the type of the disease.

This work was supported by Romanian Academy and MER-CNMP Grant No.41037.

Memory Stick

LOW-FLUENCE PDT IMPAIRS PHAGOCYTIC ACTIVITY OF HUMAN MACROPHAGES

Olga Udartseva[1,2], E. Andreeva[1,2], L. Buravkova[1], I. Andrianova[3], E. Tararak[2]

[1]Laboratory of Cell Physiology, Institute of Biomedical Problems RAS, [2]Laboratory of Molecular and Cell Cardiology, [3]Laboratory of Atherosclerosis Problems, Cardiology Research Center, Moscow, Russia

Background and objectives: Previously we have studied the susceptibility of vascular cells to photodynamic treatment (PDT) and found out that macrophages (Mph) are the most resistant and endothelial cells are the most susceptible one. So PDT will eliminate these cells first of all. However, to slow down atherosclerotic process, it's not necessary to kill cells as in tumor, but it will be sufficient to decrease their functional activity. Mph are considered to be key cells in vascular pathology because of their lipid accumulation, which is based on phagocytosis. In present study we screened effects of low-fluence PDT on Mph phagocytosis.

Methods: Human Mph were obtained and cultured as described elsewhere. 24 h before PDT 10 ug/ml sulfonated aluminium- (Photosens® (PS)) phtalocyanine was added to culture medium. Then cells were washed carefully and illuminated with 675-nm light (0,25-1 J/cm^2) (AZOR LtD, Russia). Cellular viability was measured with MTT test. To study phagocytosis latex beads were added to Mph culture medium 24 hour after PDT. In 2 hours beads were removed, cells were fixed with methanol. Quantity of phagocytes was determined using Sigma Scan software.

Results: PS accumulation alone, laser illumination alone and low-fluence PS-PDT (0,25-0,5 J/cm^2) did not affect Mph. After PDT with 1 J/cm^2 reduction of cellular viability was only 10%. Illumination of Al-sPS-loaded cells with 675-nm and 0,25-0,5 J/cm^2 fluence decreased phagocytic activity in 1,5 and 2,4 times respectively.

Conclusion: Low-fluence PDT can effectively decrease Mph phagocytic activity without reduction of other vascular cell viability.

Memory Stick

REGULATORY MECHANISMS IN CHEMOTAXIS OF DENDRITIC CELLS FROM HEALTHY BLOOD DONORS AND FROM PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

Pawel Wolkow[1], A. Gebska[1], J. Godlewski[2], J. Jawien[1], R. Olszanecki[1], M. Jawien[1], K. Zmudka[2], R. Korbut[1]

[1]Dept. of Pharmacology, [2]Dept. of Cardiology, Jagiellonian University Collegium Medicum, Krakow, Poland

Introduction: Atherosclerotic plaques contain dendritic cells (DCs), capable to initiate T lymphocyte activation. Modifiers of DC chemotaxis may affect immune response and local cytokine production, leading to plaque instability and clinical complications, e.g. myocardial infarction.

Aims of the study:

1) To determine effects of protein kinase inhibitors, angiotensin metabolites, and co - culture with thrombocytes on chemotaxis of monocyte - derived DCs from healthy blood donors.

2) To determine the effect of acute myocardial infarction on chemotaxis of peripheral blood DCs and their sensitivity to protein kinase inhibitors.

Materials and methods: Immature dendritic cells were isolated directly from peripheral blood or produced from peripheral blood monocytes by 7-day culture with GM-CSF and varying concentrations of IL-4. 2-day maturation of DCs was induced by prostaglandin E_2 with CD40 ligand or thrombocytes. Chemotaxis was induced by CCL19 in Transwell chambers. Studied substances and thrombocytes were added during maturation or shortly before the chemotaxis assay.

Results and conclusions:

1) Decrease of chemotaxis was observed only if src kinase inhibitor was added shortly before the assay, Erk kinase inhibitor during DC maturation, and p38 kinase inhibitor if DCs were cultured with low IL-4 concentration.

2) Angiotensin II inhibited and angiotensin (1-9) stimulated DC chemotaxis.

3) DCs matured in the presence of either thrombocytes or CD40 ligand have similar chemotactic potential if thrombocytes are not physically separated from DCs or treated with aspirin.

4) Chemotaxis of DCs from patients with acute myocardial infarction is impaired and their DCs are more sensitive to protein kinase inhibitors.

Memory Stick

SOME ALTERNATIVE DETERMINANTS OF VASCULAR STIFFNESS IN YOUNG INDIVIDUALS AT LOW CARDIOVASCULAR RISK

Olga Drokina[1], A. Semyonkin[1], G. Nechaeva[2], A. Zhenatov[1], L. Zhivilova[1], E. Lalyukova[2], I. Druk[2], A. Bogdalova[1], T. Miller[1]

[1]Diagnostics of Internal Diseases, [2]Postgraduate Education, Omsk Medical Academy, Omsk, Russia

Objectives: Besides cardiovascular risk factors other abnormalities may influence arterial elasticity such as genetic disorders of connective tissue as seen for example in Marfan's syndrome. Our aim was to evaluate the alternative determinants of vascular stiffness in young low risk population.

Materials and methods: The study involved 111 young healthy individuals (62 men and 49 women, 18-35 years old). Risk factors screening (blood pressure, lipids, glucose, smoking , obesity) and features of connective tissue disorders (skin, skeletal and tendons abnormalities, myopia) were performed. Stiffness index (SI) after sublingual trinitroglycerin and endothelium dependent vascular dilation (EDVD) to salbutamol were measured using photoplethysmography.

Results: SI did not differ in men and women (4.94±0.67 m/s versus 4.80±0.56 m/s, p=0.29). On multivariate analysis age, mean BP were positively and EDVD - negatively related to SI ($p< 0.005$, $p< 0.001$ and $p< 0.001$, respectively). Among connective tissue disorders features scoliosis, mild chest wall deformities, cutaneous striae and flat feet were positively related to SI ($p< 0.05$ for each variable). The model including age, mean BP, EDVD and features of connective tissue disorders that were significant on multivariate analysis accounted for 42% of SI variability ($R=0.65$, $R^2=0.42$, $p< 0,00001$).

Conclusions: Thus, as in other populations in low risk young healthy individuals vascular stiffness is determined by age, blood pressure and endothelial function. However, there exist some other factors influencing vascular elasticity, that may be related to inherited connective tissue disorders even in the absence of apparent genetic diseases such as Marfan's, Ehlers-Danlos syndrome and etc.

Memory Stick

EFFECTS OF TUMOR NECROSIS FACTOR (TNF)-ALPHA AND 17-BETA ESTRADIOL (E_2) ON PATHOLOGICAL CHANGES OF INTIMAL HYPERPLASIA

R. Nintasen[1], **Yaowapa Maneerat**[1], P. Chartiburus[2], C. Punsawad[1], V. Khachansaksumeth[1], P. Viriyavejakul[1], U. Chaisri[1]

[1]Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, [2]The Institute of Cardiovascular Diseases, Rajavithi Hospital, Bangkok, Thailand

Objectives: To compare between the effects of TNF-alpha and E_2 on pathological changes in culture of human saphenous vein with intimal hyperplasia.

Methods: A segment of saphenous vein with intimal hyperplasia was obtained from a patient at the time of bypass graft. Two millimeters thick rings of the vessel were cultured in RPMI 1640 with 30% fetal bovine serum (FBS) alone (control), plus rTNF-alpha or E_2 in static condition. After 7 days of incubation, the cultured rings were harvested to determine morphology, histopathologic changes and profiles of protein expression involving functions of endothelial cell (EC) using histomorphometric analysis, hematoxylin & eosin staining and immunohistochemistry.

Results: The rings treated with 5 ng/ml of TNF-alpha markedly increased the degree of intimal hyperplasia and proliferation of smooth muscle cells (SMC) in both tunica intima and media in comparison to those in the control rings. The damaged endothelial lining increased expression of inducible nitric oxide synthase (iNOS), and adhesion molecules including ICAM-1, VCAM-1 and E-selectin. E_2 at the dose of 10 nM could improve morphology of intimal hyperplasia observed in the control rings on day 7 of cultivation. The E_2 treated rings possessed wider lumen with perfect contour of EC lining, decreased SMC proliferation in tunica media, and reduced adhesion molecule expressions but increased endothelial nitric oxide synthase (eNOS) in EC.

Conclusion: Our finding supports previous studies in animal models that E_2 may have good efficiency to diminish degree of neointimal hyperplasia in patients who have coronary bypass graft.

Memory Stick

CAN NEUROECTODERMAL ORIGIN OF SMOOTH MUSCLE CELLS IN AORTIC ATHEROSCLEROSIS BE A PREDISPOSING FACTOR FOR THE ACCUMULATION OF LIPIDS?

Irena Tanaskovic

Department of Histology and Embryology, Medical Faculty, University of Kragujevac, Kragujevac, Serbia

Aims of this study was to examine the origine and the phenotype status of VSMCs in aortic intima.

Methods: We immunocytochemicaly examined 20 samples of atherosclerotically changed parts of the aorta descendens, at the fatty streak stage. Immunocytochemical staining (LSAB+/HRP) was performed to identify α-SMA, vimentin, and S-100 protein.

Results: In the intima of the samples analyzed in the present study, there was a substantial number of VSMCs that showed immunoreactivity to α-SMA and vimentin, some of which contain lipid inclusions in the cytoplasm (foam cells). In addition to those VSMCs, there is a presence of VSMCs that showed immunoreactivity α-SMA and S-100 protein that also look as if they are at different stages of phenotype transformations to foam cells.

Conclusions: All of the analyzed cells show a α-SMA-immunoreactivity, which indicates that we are dealing with VSMCs. Vimentin-immunoreactivity in most VSMCs point out to their syntetic/proliferative activity and also to their mesenchymal origin. It is well-known that VSMCs can also express scavenger receptors and that they transform to the foam cells. Nevertheless, in the analyzed parts of the aorta, there is a presence of foam cells that originated from VSMCs that express α-SMA and S-100 protein. The expession of S-100 protein indicates that these cells are of a neuroectodermal origine. As this protein shows a large affinity to binding unsaturated lipid acids, it has been assumed that the neuroectodermal origin of VSMCs of the coronary arteries can be a predisposing factor for the accumulation of lipids.

Memory Stick

PROTECTIVE EFFECT OF DIPHENYL DISELENIDE AGAINST PEROXYNITRITE-MEDIATED ENDOTHELIAL CELL TOXICITY: A COMPARISON WITH EBSELEN

Andreza Bem[1], P. Brito[2], P. Calcerrada[3], G. Pellufo[3], T. Dinis[2], M. Trijillo[3], J.B. Rocha[4], R. Radi[3], L. Almeida[2]

[1]Department of Biochemistry, Federal University of Santa Catarina, Florianópolis, Brazil, [2]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [3]Department of Biochemistry and Center for Free Radical and Biomedical Research, Universidad de La Republica, Montevideo, Uruguay, [4]Departament of Chemistry, Federal University of Santa Maria, Santa Maria, Brazil

Excess production of superoxide ($O_2^{-\circ}$) and nitric oxide (°NO) in blood vessel walls may occur early in atherogenesis leading to the formation of peroxynitrite, a strong oxidant and nitrating agent which represents a relevant mediator of LDL modification and of vascular injury, recognized as important steps in the atherogenic process. This study was designed to determine the effect of diphenyl diselenide $(PhSe)_2$, a new synthetic organoselenium compound, in comparison with the well known ebselen, on peroxynitrite-mediated endothelial damage. Bovine aortic endothelial cells (BAEC), were treated with authentic peroxynitrite and cell viability, intracellular gluthatione (GSH) content, gluthatione peroxidase (GPx) activity and nitroxidative stress were assessed. Additionally, the reaction kinetics of peroxynitrite with either $(PhSe)_2$ or its reduced form, phenyl selenol were explored. Experimental results showed that preincubation of endothelial cells (24 h) with low concentrations of $(PhSe)_2$ (0.5 and 1 μM) protected then from the damage promoted by peroxynitrite exposure, in a more effective way than ebselen. The intracellular levels of GSH were almost completely consumed by peroxynitrite and although the compounds did not restore the normal levels, $(PhSe)_2$ per se increased significantly GSH in a concentration-dependent manner. Moreover, $(PhSe)_2$ which revealed to be more active as a glutathione peroxidase mimic than ebselen, induced also a significantly higher increase in cellular GPx activity and protected the cells to the nitroxidative stress. The second order rate constant for peroxynitrite-mediated selenophenol oxidation was 2.7×10^5 $M^{-1}s^{-1}$. In conclusion, $(PhSe)_2$, like ebselen, shows a significant protective effect against peroxynitrite-mediated endothelial cell toxicity.

Memory Stick

CAN THE VASCULAR SMOOTH MUSCLE CELLS PLAY A ROLE OF ANTIGEN-PRESENTING CELLS IN CORONARY ATHEROSCLEROSIS?

Irena Tanaskovic

Department of Histology and Embryology, Medical Faculty, University of Kragujevac, Kragujevac, Serbia

Aim of this research has been to determine if vascular smooth muscle cells (VSMCs) can play a role in the processing and presenting of antigens to the T cells in atherosclerosis.

Methods: We examined 25 samples of atherosclerotically changed right coronary arteries, at the preatheroma stage, by transmission electron microscopy (TEM).

Results: In subendothelium of the intima of the analyzed samples we have confirmed the presence of VSMCs of the synthetic phenotype with euchromatic nucleus, a well-developed granulated endoplasmic reticulum and a reduced myofilaments, as well as a presence of foam cells (FCs), spindle-like and star-like shapes. Star-shaped FCs don´t posses basal lamina, which points out to their origine from monocyte/macrophage lineage, while spindle-shaped FCs show a smaller number of lipid droplets and a well-differentiated basal lamina, caveolae and electron dense bodies, which indicates that they originate from VSMCs. In all analyzed samples we can notice an intensive lymphocytes infiltration. A large number of lymphocytes is in nearest vicinity or direct contact both with the macrophages, and, which is especially interesting, with VSMC-s.

Conclusions: Our results could suggest that beside “professional APCs”, VSMCs also can process and present antigens. Bearing in mind that our results have shown that certain VSMCs are in direct contact with the lymphocytes, we could asssume that there is a possibility that VSMCs could display fragments of the antigens bound to the class II MHC molecule on their plasma membrane, which could cause the immunological activation of T cells and manifestation of the inflammatory reactions.

Memory Stick

CITRUS LIMON BURM F. CAN CHANGE AFFINITY OF LDL AND OX-LDL TO THEIR RECEPTORS IN RABBITS WITH ATHEROGENIC DIET

Maryam Boshtam[1], J. Moshtaghian[2], G. Naderi[1], S. Asgary[3]

[1]Laboratories, Cardiovascular Research Center/Isfahan University of Medical Sciences, [2]Animal Sciences, Sciences Faculty/University of Isfahan, [3]Basic Sciences, Cardiovascular Research Center/Isfahan University of Medical Sciences, Isfahan, Iran

Introduction: Some antioxidants can affect LDL and Ox-LDL affinity to their receptors in arteries´ endothelial. Citrus Limon Burm f. is a Citrus family member with high flavonoids(antioxidants) content that is abundant in Iran and use much by our population. We studied effect of the lemon on affinity of LDL and Ox-LDL to their receptors in rabbits on atherogenic diet.

Methods: 40 male NewZealand white rabbits were divided in four groups, normal(1) and hypercholesterolemic(2) controls, and interventions(3 and 4), randomly. Subjects' weight and serum lipids and lipoproteins were measured at the beginning and the end of the study. Except for normal control group, all rabbits put on an atherogenic diet(1% cholesterol/day) for 2 months. For group3, we added 5ml fresh lemon juice/day, and 4th group 1gr dried lemon peal powder/day during the study. At the end of the study the rabbits were sacrificed and LDL and Ox-LDL receptors of their aorta and coronary arteries were separated for each group, and the affinity of LDL and Ox-LDL to the receptors was studied by florescence absorbance(FA).

Results: FA of groups 1-4 was 8, 12, 10 and 4 for LDL receptors, and receptors was 3, 1, 7 and 7 for Ox-LDL receptors, respectively. Pathologic results show that atherosclerosis injuries are more in group 2 than 1, 3 and 4.

Conclusion: It is conclude that both Citrus Limon BURM f. peel and juice can induce LDL and reduce Ox-LDL affinity to related receptors in endothelial but the its peel does have better effect than juice.

Memory Stick

DOXYCYCLINE DOES NOT INTERFERE WITH THE POST-THROMBOTIC NEOINTIMA DEVELOPED IN AN EXPERIMENTAL MODEL SIMULATING AN AORTIC ECCENTRIC ATHEROSCLEROTIC PLAQUE

Simone G. Ramos[1], C. Tefé-Silva[1], E.M. Floriano[1], J. Elias Jr[2], M.R. Celes[1], M.A. Cortez[3], C.T. Beneli[1]

[1]Pathology, [2]Radiology, [3]Genetics, Faculty of Medicine of Ribeirão Preto/University of São Paulo, Ribeirão Preto, Brazil

Smooth muscle cell migration to form neointimas has been related to matrix degradation through the production of metalloproteinases, specifically MMP-2 and MMP-9.

Objective: We described the time sequence of post-thrombotic neointima development surrounding a hemispherical plug inserted into the aorta and discuss the participation of MMP-2 and MMP-9 in this developmental process.

Methods: *Wistar* rats were divided into four separate groups including: sham, treated-sham, operated and treated-operated groups (30/group). The treated groups were administered daily doxycycline (30mg/Kg). At days 1, 7 and 15 of the post-surgery period, aortic fragments surrounding the plug or left renal artery were excised and analyzed using optical microscopy, morphometry, immunohistochemistry, Western Blot assays, zymography and *in situ* zymography to define the neointima components.

Results and conclusions: In the operated group, the animals were characterized with mural thrombosis in the vicinity surrounding the plug 24 hours after surgery. At day seven, only loosely organized residual thrombotic fragments could be observed in the areas of blood flow recirculation and stasis that was localized both pre- and post-stenosis. At day 15, a neointima mainly composed of SMCs intermixed with extracellular matrix was formed and was superimposed to the organized thrombus. MMP-2 and MMP-9 were strongly expressed in the operated group during all periods of the study. However, the inhibition of these gelatinases in the treated group did not interfere with most of the parameters studied regarding the neointima composition. These results suggest that the SMC migration could be directly related to thrombi mediators in this experimental model.

Memory Stick

THE ROLE OF HYPOXIA IN ATHEROSCLEROSIS

Lillemor Mattsson Hultén, M. Levin, Sahlgrenska Center for Cardiovascular and Metabolic Research, Wallenberg Laboratory, Sahlgrenska Academy

University of Gothenburg, Gothenburg, Sweden

Introduction: It is important to address the factors involved in the progression of atherosclerosis because advanced atherosclerotic lesions are prone to rupture, leading to disability or death. Hypoxic areas are known to be present in human atherosclerotic lesions, and lesion progression is associated with the formation of lipid-loaded macrophages and increased local inflammation.

Recent findings: A recent study clearly demonstrated the presence of hypoxia in macrophage-rich regions of advanced human carotid atherosclerotic lesions. We showed that hypoxia increases the formation of lipid droplets in macrophages and promotes increased secretion of inflammatory mediators, and recent evidence indicates that lipid droplets may play a role in mediating the inflammatory response. Hypoxia also promotes lesion progression by exacerbating ATP depletion and lactate accumulation, and the presence of hypoxia in human carotid atherosclerotic lesions correlates with angiogenesis.

Conclusion: Recent studies indicate that hypoxia may play a key role in the progression to advanced lesions by promoting lipid accumulation, increased inflammation, ATP depletion, and angiogenesis. Further understanding of the effects of hypoxia in atherosclerotic lesions could indicate potential therapeutic targets.

Memory Stick

SYSTEM INFLAMMATION MARKERS AND MARKERS OF ENDOTHELIAL FUNCTION AT THE PATIENTS WITH CORONARY ARTERY DISEASE (UNSTABLE AND STABLE ANGINA)

Ilona Golikova

Atherosclerosis and CAD, NSC "N.D. Strazhesko Institute of Cardiology", Kyiv, Ukraine

Aim: To study the system inflammation markers and endothelial function at the patients with the stable and unstable angina pectoris. Diagnosis of coronary artery disease (CAD) confirmed by the results of coronaroangiography (CAG) with flow of disease.

Methods: 100 patients were inspected, middle ages 54,5±3,8 years. Patients were divided onto 2 groups: with the stable flow of disease (n=64) and unstable angina (n=36). All patients with the unstable angina had the signs of destruction of atherosclerotic plaque on results of CAG.

Results: The reliable increase of level of von Willebrand factor (vWF) was marked for patients with unstable angina comparing with stable patients (91,5±3,4% vs 75,4±2,8%, p< 0,05). A tendency of higher level of endothelin-1 (E-1) was found in patients with unstable angina (11,2±1,1pg/ml vs 10,6±0,6pg/m). Significant increase of inflammation markers (C-reactive protein: 20,9±2,7mg/l vs 12,3±0,8mg/l, p< 0,05; pro-inflammatory cytokines: IL-6 26,8±2,5pg/ml vs 13,8 ± 1,0pg/ml, p< 0,001; IL-8 1745±92pg/ml vs 1066±86pg/ml, p< 0,001) and decrease of antyinflammatory cytokines (IL-4 14,7 ±2,1pg/ml vs 22,4±1,6pg/ml, p< 0,05; IL-10 11,0±1,5pg/ml vs 19,0±1,4pg/ml, p< 0,05) was exposed at the patients with unstable angina also.

Conclusion: At the patients with unstable angina and angiographic signs of destruction of atherosclerotic plaque the increase activity of a system inflammation is marked and more expressed endothelial dysfunction comparing with the patients with the stable CAD.

Memory Stick

THE HAPTOGLOBIN 2-2 GENOTYPE IS ASSOCIATED WITH CAROTID ATHEROSCLEROSIS IN 64-YEAR OLD WOMEN WITH ESTABLISHED DIABETES

M. Ryndel, C.J. Behre, G. Brohall, U. Prahl, C. Schmidt, G. Bergström, B. Fagerberg, **Fredrik J. Olson**

Sahlgrenska Center for Cardiovascular and Metabolic Research, Institute of Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Introduction: Haptoglobin polymorphism generates three common human genotypes: Hp1-1, Hp2-1 and Hp2-2. Among subjects with diabetes, Hp2-2 is associated with an elevated risk to develop cardiovascular disease. The impact of haptoglobin genotype on subclinical carotid atherosclerosis is not known. We hypothesised that Hp2-2 was associated with increased occurrence of carotid atherosclerosis in subjects with diabetes.

Methods: We studied a population-based sample of 64-year old women with diabetes (*n*=226), either established diabetes known before study entry (*n*=116) or new diabetes detected at study screening. Haptoglobin genotype was determined by PCR. Carotid atherosclerosis was assessed by ultrasound imaging.

Results: In the entire diabetes cohort, no differences were observed in carotid intima-media thickness (IMT) or plaque prevalence between the genotype groups. However, among those with established diabetes, Hp2-2 was associated with higher plaque prevalence and larger carotid IMT compared with the Hp2-1 and Hp1-1 genotypes. Common cardiovascular risk factors did not differ between the genotype groups.

Conclusions: The Hp2-2 genotype was associated with increased occurrence of subclinical carotid atherosclerosis in 64-year old women with established diabetes. This association was not explained by traditional risk factors for cardiovascular disease. These results extend previous observations that Hp2-2 is associated with clinical cardiovascular disease in diabetes.

Memory Stick

NO EVIDENCE OF IMPAIRED ENDOTHELIAL FUNCTION OR ALTERED INFLAMMATORY STATE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA USING STATINS

Inger Aagnes, A. Hovland, J.H. Flage, O.-L. Brekke, K.T. Lappegård

Nordland Hospital, Bodø, Norway

Object of study: Familial hypercholesterolemia (FH) is associated with an increased risk of premature atherosclerosis; the latter is associated with inflammation and endothelial dysfunction. We wanted to investigate whether endothelial function and inflammation were altered in patients with familial hypercholesterolemia treated with statins.

Methods: Patients with FH on statins (n=14), as well as 11 healthy age- and gender-matched controls without statins participated in the study. Endothelial function was evaluated using the Endo-PAT® system from Itamar Medical Ltd, Caesarea, Israel. Fasting blood samples were drawn and 27 different cytokines, chemokines and growth factors were analyzed in addition to standard laboratory tests.

Results: There were no statistically significant differences between the FH group and the control group regarding age, weight, blood pressure or BMI. In vivo measurement of endothelial function was assessed through the Endo-PAT-system, and mean reactive hyperemia index (RHI) was 1.58 and 1.93 (p=n.s.) in the control and FH groups, respectively. There were no differences between the groups in TNF 32 vs. 26 pg/ml, IL-10 8 vs. 7, IL-6 17 vs. 9, IL-10 8 vs. 7, IL-1b 1.9 vs. 1.2, IL-1ra 88 vs. 101, MCP-1 35 vs. 45 (all p=n.s.) or any of the other inflammatory markers tested. There were no significant differences in HDL-cholesterol, LDL-cholesterol, triglycerides, APO A, APO B, Lp(a), homocysteine, HbA1c, trombocytes and fibrinogen between the groups.

Conclusions: Endothelial function and inflammatory state assessed by RH-PAT or inflammatory biomarkers were not different in FH patients on statins compared to healthy controls.

Memory Stick

LONG-TERM EFFECT OF STATINS ON THE RISK OF NEW-ONSET DIABETES - A RETROSPECTIVE COHORT STUDY

Gwo-Ping Jong[1], L. Tien[2], T. Ma[3]

[1]Division of Cardiology, Armed Forces Taichung General Hospital, [2]Central Region Branch, Bureau of National Health Insurance, [3]Department of the Health Service Management, China Medical University, Taichung, Taiwan R.O.C.

Statins have been linked to new-onset diabetes (NOD); however, data on the effect of these drugs on the development of NOD in hyperlipidemic patients has not been well determined. We aimed to investigate the association between statins and NOD. This was a retrospective cohort study performed using data from claim forms provided to the central region branch of the Bureau of National Health Insurance in Taiwan from January 2004 to December 2008. Prescriptions for statins before the index date were retrieved from a prescription database. We estimated the hazards ratios (HRs) of NOD associated with statins use; nondiabetic subjects served as the reference group. A total of 1250 NOD cases were identified in 19014 hyperlipidemic patients during the study period. The risk of NOD after adjusting sex and age was higher among users of lovastatins (HR, 1.22; 95% confidence interval (CI), 1.07-1.38) than among non-users. Patients who take fluvaststins (HR, 0.61; 95% CI, 0.44-0.84) are at a lower risk of developing NOD than non-users. Pravastatins, simvastatins, and atorvastatins were not associated with risk of NOD. The results of this study suggest that hyperlipidemic patients who take fluvaststins are at lower risk of NOD. Lovastatins were associated with a significant increase in the risk of NOD.

Memory Stick

ADULTHOOD TELEVISION VIEWING RELATES INDEPENDENTLY TO CARDIOMETABOLIC RISK PROFILE IN EARLY MIDDLE AGE

Emmanuel Stamatakis[1], M. Hamer[1], G. Mishra[1,2]

[1]Epidemiology & Public Health, University College London, [2]Medical Research Council, London, UK

Introduction: There is little research examining the longitudinal associations of sedentary behaviour and cardiovascular risk.

Objective: To examine the independent associations between TV viewing in early adulthood and cardiometabolic risk in middle age.

Design: The study sample comprised 5629 members (2683 men) of the 1958 British Birth Cohort. TV watching / exercise frequency were measured at age 23 and daily TV and weekly moderate to vigorous physical activity (MVPA) at age 44, as well as 15 biological cardiometabolic risk factors. Factor analysis revealed three risk factor components (explaining 57% of the risk variance: a metabolic (C1: triglycerides, HDL, BMI, waist, blood pressure), a haemostatic/inflammatory (C2: fibrinogen, von Willebrand factor, d-dimer, c-reactive protein), and a cholesterol (C3: total cholesterol, LDL).

Results: TV at age 23 showed strong multivariable-adjusted associations with C1 (: Generalized linear model coefficient : 0.26, 95%CI: 0.16 to 0.37, p< 0.001), C2 (0.20, 0.9 to 0.32, p< 0.001), but not with C3 (0.05, -0.17 to 0.1, p=0.9). For C1 and C2, associations remained strong following adjustments for physical activity and TV watching at age 44 (C1: 0.17, 0.01 to 0.29, p< 0.001; C2: 0.13, 0.02 to 0.25, p=0.01). Among physically active participants, TV watching had linear associations with C1 (0.14, 0.02 to 0.25, p=0.02) and C2 (0.23, 0.11 to 0.35, p< 0.001).

Conclusions: TV watching during early to mid adulthood was associated independently with the metabolic and haemostatic risk factor profiles at age 44.

Memory Stick

TF AND TFPI POLYMORPHISMS IN CORONARY HEART DISEASE AND DIABETES TYPE 2

Trine Opstad[1], A.Å. Pettersen[1], S. Åkra[1], T. Weiss[1], H. Arnesen[1,2], I. Seljeflot[1,2], Center for Clinical Heart Research

[1]Department of Cardiology, Center for Clinical Heart Research, Oslo University Hospital, Ulleval, [2]University of Oslo, Oslo, Norway

Introduction: Tissue factor (TF) and its endogenous inhibitor (TFPI) are the main regulators of the initiation of the coagulation process, important in atherothrombosis.

Objectives: To compare the frequency of six known single nucleotide polymorphisms (SNPs) in the genes coding for TF and TFPI in CHD patients as compared to healthy individuals. Genotypes and phenotypes were evaluated with special emphasis on diabetes and gender differences in the CHD population.

Results: No significant differences in frequencies between the CHD population and the healthy controls of any polymorphisms were observed. In the CHD population, the TF 5466 AG/GG genotypes were significantly more frequent in women as compared to men ($p < 0.001$). The two linked SNPs, the TF-1812CT/TT and the TF-603AG/GG genotypes were significantly more frequent in women without diabetes type 2 as compared to women with diabetes ($p < 0.018$, both).

In the total CHD population, the TF-1812CT and the TF-603AG genotypes were associated with significantly lower sTF levels as compared to the homozygous genotypes ($p < 0.02$, both). The TFPI-399CT/TT and the TFPI-33TC/CC genotypes were associated with lower and higher TFPI total antigen levels, respectively, as compared to their respective TFPI-399CC and TFPI-33TT genotypes ($p < 0.001$, both). Women with diabetes type 2 presented lower TFPI total antigen, statistically significant only for the TFPI-399CC and the TFPI-287TT genotypes.

Conclusion: Genetic variations in the TF gene seem to be influenced by gender. Women presenting with diabetes type 2 showed a different pattern in TF and TFPI genotypes, which to some degree associated to their respective phenotypes.

Memory Stick

BIOMARKERS OF ATHEROGENESIS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND THEIR CORRELATION WITH DISEASE ACTIVITY

Narayan Bhowmik

Dept. of Critical Care Medicine, BIRDEM/BSM Medical University, Dhaka, Bangladesh

Introduction: Epidemiological studies have revealed an increased mortality due to cardiovascular (CV) complications in patients with RA. Patients with this disease have an increased risk of cardiovascular death and congestive heart failure that is unrelated to the presence of traditional atherosclerosis risk factors. Endothelial dysfunction, an early step in the atherogenesis process, is observed in both early and long-standing actively treated patients with RA.

Objectives: To assess the status of biomarkers of atherogenesis in patients of rheumatoid arthritis (RA) and their relation with RA disease activity.

Materials and methods: Consecutive forty- five patients fulfilling ACR 1987 criteria for RA were enrolled from Rheumatology wing, BSM Medical University of Bangladesh. Forty two healthy individuals enrolled as control.

parameters as measure of disease activity and biomarkers of atherogenesis (FSG, FSI, Triglyceride, Cholesterol, LDL, HDL, ESR, High-sensitivity CRP, tHcy, Vitamin B12, Folate, Creatinine and CCR) studied and analyzed.

Results: There are significant correlations between clinical parameters of RA disease activity and proatherogenic biomarkers in RA patients.**Conclusions:**

1. The levels of the biomarkers: FSG, FSI, TG, Cholesterol, LDL, HDL, ESR, High-sensitivity CRP, Creatinine and CCR were significantly altered in favor of accelerated atherogenesis in rheumatoid arthritis patients.

2. There are significant correlations between FSG, FSI with swollen joint count (SJC), painful joint count (PJC), global disease activity on VAS of patient (GVASP), global disease activity on VAS of doctor (GVASD).

Memory Stick

THE VITAMIN E BINDING PROTEIN AFAMIN IS ASSOCIATED WITH THE METABOLIC SYNDROME AND INFERTILITY

Hans Dieplinger[1], G. Wietzorrek[2], S. Olscher[1], S. Kiechl[3], J. Willeit[3], G. Wakonigg[4], W. Engel[5], F. Kronenberg[1]

[1]Genetic Epidemiology, [2]Pharmacology, [3]Neurology, Innsbruck Medical University, [4]Vitateq Biotechnology, Innsbruck, Austria, [5]University of Goettingen, Goettingen, Germany

The pathogenesis of the metabolic syndrome is multifactorial and polygenic. Several heritability studies indicated a major role of genetic susceptibility for the metabolic sndrome.

1-year old transgenic mice overexpressing the gene for the vitamin E-binding protein afamin had significantly higher body weight and plasma concentrations of glucose, cholesterol and triglycerides compared to their wild-type littermates suggesting a role of afamin in carbohydrate and lipid metabolism. To test whether these findings hold true also for human populations, we measured afamin plasma concentrations in the prospective population-based Bruneck study (n=826) and found significant associations with waist-to-hip ratio, body mass index, obesity, systolic and diastolic blood pressure, diabetes, and plasma concentrations of LDL- and HDL-cholesterol, triglycerides, free fatty acids, glucose and Hba1c. In addition, afamin concentrations were also positively correlated with increasing numbers of these parameters in a 10 years follow-up prospective observation.

These results (particularly those from transgenic mice and prospective observations) indicate not only an association between afamin and the metabolic syndrome but suggest causality and a high predictive potential of afamin for developing this modern epidemic disease.

In contrast, genetically modified mice in which the afamin gene was deleted were infertile already at the chimeric (heterozygous) level. Male animals exhibited a grossly altered testis histology showing severely degenerated testis tissue and almost absent spermiogenesis. Application of exogenous recombinant mouse afamin (via implanted diffusion pump) could completely restore testes tissue histology and fertility.

In summary, afamin seems to play major roles in the development of cardiovascular and infertility disorders.

Memory Stick

SYSTEMIC LEVELS OF METALLOPROTEINASE-2/ITS INHIBITOR SYSTEM ARE ASSOCIATED WITH QUINOLINIC ACID LEVELS IN PATIENTS WITH CARDIOVASCULAR DISEASE ON PERITONEAL DIALYSIS

Krystyna Pawlak[1], J. Tankiewicz[2], M. Mysliwiec[3], D. Pawlak[1]

[1]Monitored Pharmacotherapy, [2]Cardiology, [3]Nephrology and Clinical Transplantation, Medical University, Bialystok, Poland

Objective: Matrix metalloproteinases (MMPs), their inhibitors (TIMPs), oxidative stress (SOX) and kynurenine (KYN) pathway have been postulated in cardiovascular disease (CVD) progression. We hypothesized the possible association between MMPs/TIMPs system, kynurenines and CVD prevalence in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: We assessed MMP-2, MMP-9, TIMP-1, TIMP-2, KYN and its metabolite - quinolinic acid (QA), and SOX marker - Cu/Zn superoxide dismutase (Cu/Zn SOD) levels in CAPD patients both with and without CVD and healthy controls.

Results: MMP-2, TIMP-2, Cu/Zn SOD, KYN and QA were significantly higher in CAPD patients with CVD than in patients without CVD and controls. Both MMP-2 and TIMP-2 were positively correlated with QA and Cu/Zn SOD levels, and the strong association was between MMP-2 and TIMP-2 levels. Multiple regression analyses identified Cu/Zn SOD, TIMP-2, QA and QA/KYN ratio as the factors independently associated with MMP-2, whereas MMP-2 and Cu/Zn SOD were independent variables affecting TIMP-2 levels.

Conclusions: MMP-2 and TIMP-2 concentrations were higher in CAPD patients with CVD than in patients without CVD and healthy controls. Up-regulation MMP-2/TIMP-2 system was associated with QA levels and increased oxidative status, suggesting the connection between KYN pathway activation, arterial remodeling, oxidative stress and CVD prevalence in uremic patients on CAPD treatment.

Memory Stick

ASSOCIATION OF TOLL-LIKE RECEPTOR-6 PRO249SER POLYMORPHISM WITH DECREASED CYTOKINE PRODUCTION AND LOWER LEFT VENTRICULAR WALL THICKNESS IN HYPERTENSIVE WOMEN

Roberto Schreiber[1], M.L. Sales[2], M.N. Fernandes[1], M.C. Ferreira-Sae[1], J.A. Cipolli[1], C. Piveta[1], J.R. Matos-Souza[1], A. Calixto[1], B. Geloneze[1], K.G. Franchini[1], W. Nadruz Jr[1]

[1]Department of Internal Medicine, School of Medical Sciences, State University of Campinas, Campinas, [2]Department of Internal Medicine, School of Medical Sciences, Ouro Preto University, Ouro Preto, Brazil

Background and aims: Experimental data demonstrated that inactivation of Toll-Like Receptor (TLR) pathway components attenuated left ventricular (LV) hypertrophy induced by pressure overload. This study investigated the impact of TLR6 Pro249Ser polymorphism on LV structure in hypertensive subjects.

Methods: We evaluated 392 patients (238 women and 154 men) by clinical history, physical examination, anthropometry, analysis of inflammatory and metabolic parameters and echocardiography. The polymorphism was analyzed by polymerase chain reaction and digestion with restriction enzyme. Furthermore, we investigated whether monocytes from TLR6 249Ser/Ser subjects presented a defective response to stimulus with the TLR6 agonist zymosan. Data are presented as the mean ± standard error.

Results: Homozygous women for the TLR6 249Ser allele (n=12) had lower LV posterior wall thickness (9.4±0.4 vs 10.5±0.1mm; p=0.02), interventricular septum thickness (9.7±0.3 vs 10.7±0.1mm; p=0.02) and LV relative wall thickness (0.38±0.02 vs 0.44±0.01; p=0.02) than women with the Ser/Pro and Pro/Pro genotypes. These results were confirmed by stepwise regression analyses adjusted for systolic and diastolic blood pressure, age, body mass index, diabetes mellitus, menopause status and use of antihypertensive medications. Conversely, homozygous men for the 249Ser variant showed no differences in LV structure in comparison to males carrying the 249Pro allele. In addition, monocyte-derived macrophages with the 249Ser/Ser genotype from women, but not from men, presented reduced zymosan-induced release of interleukin-6 and tumor-necrosis factor-alpha.

Conclusion: These data indicate that hypertensive women homozygous for the TLR6 249Ser polymorphism exhibit reduced TLR6 protein function and lower LV wall thickness in comparison to those carrying the 249Pro allele.

Memory Stick

PARENTAL PREDICTORS OF METABOLIC SYNDROME (JOINT INTERIM STATEMENT 2009 CRITERIA) IN ADULT CHILDREN OF PATIENTS WITH PREMATURE CORONARY HEART DISEASE

Michail Konnov, L. Dobordzhginidze, A. Deev, N. Gratsiansky

Laboratory of Clinical Cardiology, Institute of Physico-Chemical Medicine, Moscow, Russia

Purpose: To elucidate associations between metabolic syndrome (MS, Joint Interim Statement 2009 criteria) in adult children of patients with premature coronary heart disease (PCHD) (onset < 55, men; < 60 years, women) with parental and own characteristics.

Methods: We examined 198 families - 188 parents-probands (121 men) aged 36-67 years, their 126 consorts (8 with CHD, 32 men) aged 36-67 years, 236 their children without vascular disease (124 men) aged 16-37 years. Characteristics included alcohol, smoking, contraceptives, height, BMI, waist circumference (WC), heart rate (HR), systolic/diastolic BP, total-, HDL-, LDL cholesterol, triglycerides, serum glucose, prehypertension/hypertension (NHBPEP-4/JNC-7 age dependent criteria), impaired glucose tolerance, diabetes (DM), MS and (for parents) education and menstruation status. Predictors of MS were selected by sex, age adjusted logistic regression analysis.

Results: MS was found in 115/188 probands (66 men), 57/126 consorts (12 men) and 31/236 children (21 men). At univariate logistic regression analysis children's MS was associated only with their sex: OR sons vs. daughters was 2.54 (95% confidence interval [CI] 1.10-5.84, p=0.029). Parental characteristics related to MS in univariate analysis with p< 0.1 (DM, WC, HR, MS of proband, DM, BMI, WC, glucose, MS of consort) were included into stepwise regression procedure. Factors independently linked with children´s MS were MS of consort (OR 21.1, 95%CI 2.57-174.2, p=0.0046); and DM of proband (OR 2.60, 95%CI 1.04-6.51, p=0.040).

Conclusion: In this group of children of PCHD patients: sons were more prone to MS; MS of parent-consort and (weaker) DM of parent-proband were characteristics independently associated with MS.

Memory Stick

BODY FAT RELATED TO RISK FACTORS FOR CVD IN YOUNGER CHILDREN

Magnus Dencker[1], O. Thorsson[1], M.K. Karlsson[2], C. Lindén[2], P. Wollmer[1], L.B. Andersen[3]

[1]Dept of Clinical Sciences, Unit of Clinical Physiology and Nuclear Medicine, [2]Dept of Clinical Sciences, Clinical and Molecular Osteoporosis Research Unit, Malmö University Hospital, Lund University, Malmö, Sweden, [3]Institute of Sport Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark

Introduction: Obesity is associated with increased risk of cardiovascular disease (CVD) in adults. We analysed if body fat is related to clustering of other risk factors for CVD in children aged 8 to 11 years.

Methods: Cross-sectional study of 243 (136 boys and 107 girls) children aged 8-11 years, recruited from a population-based cohort. Total fat mass was measured by Dual-energy x-ray absorptiometry and expressed as percentage of total body mass (BF%). Maximal oxygen uptake (VO_{2PEAK}) was assessed by indirect calorimetry during maximal exercise test. Systolic and diastolic blood pressure (SBP, DBP), and resting heart rate (HR) were measured. Echocardiography, 2-dimensional guided M-mode, was performed in accordance with ASE guidelines and left ventricular mass (LVM) was calculated and adjusted for height. Z-scores (Value for the individual-mean value for group)/SD were calculated. Sum of z-scores for SBP, DBP, HR, LVM, and -VO_{2PEAK} were calculated in boys and girls, separately, and used as indices of clustered risk.

Results: Mean BF% was 18.8±9.2% (range 6.2-45.7%). Pearson correlation between ln BF% versus indices of clustered risk was for boys ($r=0.55$, $P< 0.05$), and for girls ($r=0.39$, $P< 0.05$). Boys and girls were divided according to tertiles of BF%. One-way ANOVA analysis indicated highly significant differences in sum of z-scores between tertiles of BF% in boys ($P=< 0.001$), and in girls ($P=< 0.001$).

Conclusion: Findings from this population-based cohort of young children shows that BF% was associated with a clustering of other cardiovascular risk factors.

Memory Stick

COMPARISON OF RISK FACTOR GRAPH VERSUS FRAMINGHAM RISK SCORE IN PREDICTING THE POPULATION AT RISK OF ATHEROTHROMBOTIC DISEASE

William Feeman, Jr

The Bowling Green Study, Bowling Green, OH, USA

Introduction: The Framingham Risk Score (FRS) is known to be a poor predictor of atherothrombotic disease (ATD) in women and to misclassify ATD risk in men.

Objective: To show that a multifactorial risk factor graph is a superior predictor to the FRS.

Methods: The Bowling Graeen Study (BGS) has an age-sex data base of 709 patients who suffered an ATD event in the 1974-2003 timeframe. The BGS graph was created to identify those ATD risk factors that best defined the population who eventually developed ATD. The BGS graph utilizes the Cholesterol Retention Fraction (CRF, or [LDL-HDL]/LDL on the ordinant and systolic blood pressure (SBP)on the abcissa. When combined with cigarette smoking status, this graph accurately identifies the population at risk of ATD. Only 6% of ATD patients could not be identified by CRF-SBP plot position above the graph´s threshold line and/or cigarette smoking status.

Results: In males the BGS graph and FRS agreed in 85% of cases; in event of disagreement, the BGS predicted the younger males missed by the FRS. In women, the BGS and FRS agreed in only 40% of cases; in event of disagreement, the FRS missed assigning risk all across the age spectrum.

Conclusions: The BGS graph provides a superior means of predicting the population at risk of ATD, when compared to the FRS.

Memory Stick

THE RELATION BETWEEN ARTERIAL STIFFNESS AND AUTONOMIC NERVOUS FUNCTION CLASSICAL TESTS IN TYPE 2 DIABETIC PATIENTS

Dimtrios Levisianou[1], A. Melidonis[1], S. Foussas[1], E. Adamopoulou[1], A. Koutsovasilis[1], T. Xenopoulou[2], G. Koukoulis[3], I. Skoularigis[3], F. Triposkiadis[3]

[1]Tzanio General Hospital, Piraeus, [2]Agios Panteleimon General Hospital, Nikea, [3]University of Thessaly, Larissa, Greece

Background and aims: Arterial Stiffness, increased in people with diabetes, reflects the grade of atherosclerosis and consists a well-established cardiovascular risk factor. Diabetic autonomic neuropathy, is correlated with increased cardiovascular mortality. There are studies that were prove correlation between these two risk factors. We sought to find out the relation between arterial stiffness and cardiac autonomic neuropathy according to ADA and AAN consensus statement.

Materials and methods: Forty people with type 2 diabetes underwent in the 4 classical autonomic nervous function (expiration/inspiration index, valsava index, 30:15 index and variation of systolic blood pressure index). Arterial stiffness was estimated by measuring carotid femoral pulse wave velocity.

Results: With arterial stiffness as a dependent variable and ANF test as independent variables, Spearman's correlation coefficient (r_s) was for E/I index 0.031(p=0.211), for 30:15 index 0.564(p=0.020), for valsava index 0.028(p=0.218) and for SBP index 0.295(p=0.234). Using Bonferroni's test there was statistical significant differences in cf-PWV between subjects with three abnormal AFN tests and those with one abnormal test (p=0.035). There was no statistical significant difference between patients with one abnormal AFN test and two or patients between two and three abnormal tests**.**

Conclusions: From our results we conclude that arterial stiffness isn´t correlated to CAN according ADA and AAN consensus statement. On the contrary cf-PWV is independently correlated 30:15 index but not with any other of the rest AFN tests. Additionally the increment of abnormal AFN tests, which are present in a diabetic patient, reflects respective increment in cf-PWV that means even stiffer arteries.

Memory Stick

THE INFLUENCE OF APOLIPOPROTEIN E GENETIC VARIANTS ON SERUM LIPIDS AND INSULIN RESISTANCE IN TARF STUDY

Evrim Komurcu Bayrak[1], A. Onat[2], B. Yuzbasiogullari[1], N. Mononen[3], R. Laaksonen[3], G. Hergenc[4], S.E. Humphries[5], T. Lehtimäki[3], N. Erginel-Unaltuna[1]

[1]Department of Genetics, Institute for Experimental Medicine, Istanbul University, [2]Emeritus, Department of Cardiology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey, [3]Department of Clinical Chemistry, Tampere University Hospital and University of Tampere, Medical School, Tampere, Finland, [4]Emeritus, Department of Biology, Yildiz Technical University, Istanbul, Turkey, [5]Centre for Cardiovascular Genetics, Rayne Institute, Royal Free and University College London (UCL) Medical School, London, UK

Objective: The apoE ε2/ε3/ε4 polymorphism is one of the most thoroughly studied polymorphisms and it was related with lipids as well as coronary artery disease. In addition, the -219G/T and +113G/C polymorphisms within the regulatory region were related with transcriptional activity of the apoE gene. We, therefore, examined the effect of -219G/T and +113G/C polymorphisms and the apoE haplotypes based on the ε2/ε3/ε4 polymorphism with lipids and insulin resistance in Turkish Adult Risk Factor (TARF) Study.

Methods: A population-based cross-sectional survey conducted from 1998-2008 included 1774 randomly selected adults (mean age 55.03±11.7 years, 51.2% women) participating in the TARF Study. We analyzed -219G/T, +113G/C and ε2/ε3/ε4 polymorphisms as well as their haplotypes. Insulin resistance was defined by the upper 70th percentile in the sample (>2.512) of the homeostatic model assessment (HOMA) index.

Results: The ε2-allele, ε3-allele, ε4-allele, -219 T-allele, and +113 C-allele frequencies in adult Turkish population were 0.055, 0.871, 0.074, 0.477, and 0.423, respectively. The haplotype1 (GGε3) and haplotype2 (TCε3) were 44.1% and 41.9%, respectively. The ε2/ε3/ε4 polymorphism showed strongly associations with the serum the total cholesterol, HDL-C, LDL-C, apo B and apo E levels ($p< 0.001$). The SNPs -219G/T and +113G/C were in association with fasting insulin levels and HOMA index ($p< 0.05$), but no association with lipids. The diplotypes of the haplotype2 (TCε3) were associated with insulin levels, HOMA index and insulin resistance ($p< 0.05$).

Conclusion: ApoE allelic variant is related with serum insulin levels and HOMA index as well as insulin resistance in Turkish adults.

Memory Stick

LOW BONE MINERAL DENSITY IS NOT ASSOCIATED WITH ANGIOGRAPHICALLY DETERMINED CORONARY ATHEROSCLEROSIS IN MEN

Stefan Beer[1,2,3], C.H. Saely[1,2,3], G. Hoefle[1], P. Rein[1,2,3], A. Vonbank[1,2,3], J. Breuss[1], B. Gaensbacher[1], A. Muendlein[1,3], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: The association of low BMD with angiographically determined coronary atherosclerosis in men is unknown.

Methods: We enrolled 623 consecutive men undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD). BMD was assessed by dual X-ray absorptiometry. CAD was diagnosed in the presence of any coronary artery lumen narrowing at angiography; coronary stenoses with lumen narrowing ≥50% were considered significant.

Results: From the total study cohort (mean age of 64±11 years), 207 patients (33.2%) had osteopenia and 65 (10.4%) had osteoporosis; at angiography, CAD was diagnosed in 558 patients (89.6%) and 403 (64.7%) had significant coronary stenoses. In multivariate logistic regression analysis neither osteopenia nor osteoporosis was associated with an increased prevalence of CAD (adjusted odds ratios (ORs) = 0.71 [95% confidence interval 0.40-1.23]; p = 0.222 and 1.03 [0.38-2.80]; p = 0.955, respectively) or with significant coronary stenoses (OR 0.74 [0.52-1.07], p = 0.112 and 0.72 [0.41-1.26]; p = 0.251, respectively). Also as a continuous variable BMD was not associated with angiographically diagnosed CAD.

Conclusions: The prevalence of low BMD is very high in men undergoing coronary angiography. However, low BMD is not associated with angiographically determined coronary atherosclerosis in men.

Memory Stick

ARTERIAL STIFFNESS IS ASSOCIATED WITH LEFT VENTRICULAR FUNCTION IN PATIENTS WITH HYPERTENSION ARTERIAL AND DYSLIPIDAEMIA

Lucia Agoston-Coldea, T. Mocan

"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Objective: To evaluated relationship between arterial stiffness and left ventricular diastolic dysfunction in patients with hypertension arterial and dyslipidaemia with preserved left ventricular ejection fraction.

Methods: Sixty six patients with hypertension and dyslipidaemia (240 mg/dL for plasma total cholesterol) were randomized to either administration of atorvastatin (20 mg/day; n=36) or no statin therapy (n=30) for 6 months. Left ventricular systolic and diastolic functions were evaluated by measuring transmitral flow velocity, mitral annular motion velocity, and the myocardial strain and strain rate profiles. Subclinical atherosclerosis also was determined by measuring the intimae - media thickness and stiffness carotid arteries.

Results: The mean peak early diastolic strain rates of the left ventricular posterior and inferior walls also increased from -5.9±1.6 s-1 to -8.2±1.5 s-1 ($p< 0.05$) and -5.1±1.2 s-1 to -7.8±1.8 s-1 ($p< 0.05$), respectively, in the atorvastatin group. The stiffness carotid arteries correlated with the peak early diastolic velocity of transmitral flow velocity ($r=-0.48$, $p< 0.05$), the ratio of peak early to late diastolic transmitral flow velocity ($r =-0.41$, $p< 0.05$), the deceleration time from peak to baseline of the early diastolic transmitral flow velocity ($r=0.60$, $p< 0.05$), the peak early diastolic mitral annular motion velocity ($r=-0.45$, $p< 0.05$), and the peak early diastolic strain rates at the endocardial sites of the left ventricular posterior and inferior walls ($r=0.64$, $p< 0.05$; $r=0.52$, $p< 0.05$, respectively).

Conclusions: Atorvastatin improved not only carotid arterial stiffness but also regional left ventricular systolic and diastolic function in patients with hypertension.

Memory Stick

GENETIC VARIANT RS4355801 A>G IS ASSOCIATED WITH BOTH ANGIOGRAPHICALLY DETERMINED CORONARY ATHEROSCLEROSIS AND BONE MINERAL DENSITY

Christoph H. Saely[1,2,3], S. Beer[1,2,3], A. Muendlein[1,3], A. Vonbank[1,2,3], P. Rein[1,2,3], J. Breuss[1], B. Gaensbacher[1], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: A recent genome-wide association study found evidence for an association between bone mineral density (BMD) and variant rs4355801 on chromosome 8, near to the osteoprotegerin gene. Associations between bone mineral density (BMD) and atherosclerotic disease have been suggested. Potential links between variant rs4355801 and coronary artery disease (CAD) are not known.

Methods: We performed genotyping of variant rs4355801 in a large cohort of 1593 consecutive Caucasian patients undergoing coronary angiography for the evaluation of established or suspected stable CAD; significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥50%. BMD of lumbar spine and femur was by assessed by Dual Energy X-ray Absorptiometry (DXA) in a subset of 823 subjects.

Results: The prevalence of significant CAD increased significantly from the AA over the AG to the GG genotype (55.0%, 57.6%, and 64.2%, respectively; p_{trend}=0.011). The odds ratio for homozygous carriers of the G allele vs. carriers of the A allele was 1.37 [95% CI 1.06-1.79] after adjustment for age and gender. Further, BMD scores increased significantly from the AA over the AG to the GG genotype (1.10±0.20, 1.13±0.20, and 1.16±0.19, p_{trend}< 0.001 and 0.96±0.18, 1.00±0.15, and 1.02±0.15 p_{trend}< 0.001, respectively).

Conclusions: Genetic Variant rs4355801 A>G is associated with both angiographically determined coronary atherosclerosis and BMD, pointing to a pathophysiological link between CAD and bone mineralisation.

Memory Stick

GENETIC VARIANT RS1051730 C>T IN THE NICOTINIC ACETYLCHOLINE RECEPTOR GENE CLUSTER ON CHROMOSOME 15Q24 SIGNIFICANTLY PREDICTS SMOKING SEVERITY IN CORONARY

Christoph H. Saely[1,2,3], A. Muendlein[1,3], A. Vonbank[1,2,3], P. Rein[1,2,3], S. Beer[1,2,3], J. Breuss[1], B. Gaensbacher[1], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University in the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Smoking is a major cause of preventable premature death, mainly due to its strong and dose-dependent impact on coronary artery disease (CAD). Recently, genetic determinants of nicotine dependence (which correlates with the amount of smoked cigarettes rather than with the smoker status per se) have been suggested. No data are available for patients already affected by CAD.

Methods: We genotyped variant rs1051730 C>T in the nicotinic acetylcholine receptor gene cluster on chromosome 15q24 in a large cohort of 1303 consecutive Caucasian patients with angiographically proven CAD.

Results: From our patients, 62.1% had a history of smoking (n = 809; 226 current and 581 past smokers). Genotype distributions of variant rs1051730 were not significantly different between patients with a history of smoking and those who had never smoked (p_{trend} across genotypes = 0.471). However, the variant among smokers proved strongly predictive for the average amount of cigarettes smoked per day (24/d, 23/d, and 30/d for subjects with the AA, the AT, and the TT genotypes; $p < 0.001$ for those with the TT genotype vs. carriers of the A allele). This association remained significant after adjustment for age and gender ($F = 12.4$; $p < 0.001$).

Conclusions: Genetic variant rs1051730 C>T in the nicotinic acetylcholine receptor gene cluster significantly predicts smoking severity in patients with angiographically proven CAD.

Memory Stick

IMPACT OF MATERNAL AND PATERNAL HISTORY OF PREMATURE MYOCARDIAL INFARCTION ON THE AGE OF MANIFESTATION OF ACUTE CORONARY SYNDROME.

Jan Pitha[1], V. Stanek[1], R. Poledne[1], M. Gebauerova[1], M. Aschermann[2], H. Skalicka[2], J. Matouskova[3], A. Kruger[3], M. Penicka[4], H. Hrabakova[4], J. Veselka[5], P. Hajek[5]

[1]Institute for Clinical and Experimental Medicine, [2]General Teaching Hospital, [3]Homolka Hospital, [4]University Hospital Kralovské Vinohrady, [5]University Hospital Motol, Prague, Czech Republic

Introducion: Family history is a strong and independent risk factor for acute coronary syndrome (ACS), especially at younger age. We studied the impact of maternal and paternal history of premature myocardial infarction (MI) on the age of manifestation of ACS.

Methods: We evaluated the association between maternal and paternal history of premature MI and the age of manifestation of ACS in 1,063 men and in 415 women with ACS admitted to 5 coronary care units in Prague. Maternal history was defined as fatal/nonfatal MI in mother before age of 65 (found in 45 men and in 28 women); paternal history as fatal/nonfatal MI in father before age of 60 (found in 131 men and in 45 women). Differences were analyzed by unpaired t-test (STATA).

Results: Men with maternal history of MI were 2 years younger (borderline significance) than those without maternal history of MI (53.1±7.4 vs. 55.3 ±7.5 years; p=0.055); even reverse (non-significant) trend was found in women with and without maternal history of premature MI (64.0±8.0 vs. 62.5±8.6 years; p=0.30). In contrast, men with paternal history of MI were significantly younger by more than 3 years than those without paternal history of MI (52.4±8.5 vs. 55.6 ±7.3 years; p< 0.001) and even higher difference (more than 5 years) was found in women (57.9±11.3 vs. 63.2 ±8.0 years; p< 0.001).

Conclusions: Paternal but not maternal history of MI predicted younger age of manifestation of ACS. The paternal history might be stronger risk factor for younger age of ACS in women.

Memory Stick

PERCEPTION OF THE RELEVANCE OF LIPID PARAMETERS AND TARGET VALUES ESTIMATED BY PHYSICIANS IN EUROPE/CANADA: LESSONS LEARNED FROM DYSIS

Wenefrieda Smolka[1], C. Juenger[2], K. Bestehorn[1], A.K. Gitt[2], on behalf of DYSIS Investigators

[1]MSD Sharp & Dohme GmbH Haar, Haar, [2]Institut fuer Herzinfarktforschung Ludwigshafen an der Universitaet Heidelberg, Ludwigshafen, Germany

Aims: Overwhelming evidence exists of the efficacy of lipid lowering treatment with statins on the prevention of cardiovascular events. Guidelines recommend treatment targets/normal levels for different lipid parameters. Little is known about the physicians` perception of these recommendations in clinical practice.

Methods: DYSIS (Dyslipidemia International Study) was an epidemiological cross-sectional study to assess the lipid profile and patients´ characteristics of 22,063 consecutive statin-treated patients during a single visit to their physicians (2987 centres) on outpatient basis in Europe and Canada. Treating physicians were asked for their relevance valuation of lipid parameters and the corresponding target value for each patient.

Results: In European countries and Canada LDL-C was perceived as the most relevant lipid parameter but LDL-C target values were higher in European countries vs. Canada (p< 0.0001). Details are displayed in the table.

	LDL-C relevance	LDL-C target	HDL-C relevance	HDL-C target	TG relevance	TG target
Europe	87	100/2.6	72	45/1.2	68	150/1.7
Canada	96	77/2.0	71	40/1	66	152/1.7
Relevance (took into account parameter in management of lipids),in %; LDL-C (low density cholesterol), HDL-C (high density cholesterol), TG (triglycerides) [mg/dL /mmol/L]						

[Table]

Conclusion: LDL-C is highly relevant to physicians; targeted values were higher (23%) in Europe vs. Canada. HDL-C and TG are consistently of lower relevance to treating physicians. Target values of HDL-C assigned by physicians were higher in Europe vs. Canada. Target values of TG didn´t differ. High awareness exist to capture lipid disorders by LDL-C data and less awareness exist for HDL-C and TG when determining mixed dyslipidemia.

Memory Stick

LUPINE KERNEL FIBRE CAN HELP TO MANAGE RISK FACTORS FOR ATHEROSCLEROSIS

Anita Fechner, G. Jahreis

Department of Nutritional Physiology, Institute of Nutrition; Friedrich Schiller University Jena, Jena, Germany

There is evidence that the consumption of legume kernel fibre, containing both soluble and insoluble fibre fractions, may beneficially modify coronary and colonic health.

Therefore, the objective of two human intervention studies was to determine the efficacy of a native lupine kernel fibre (*L. angustifolius* Boregine*)* on the prevention of risk factors for gastrointestinal or cardiovascular diseases.

In study I 26 healthy subjects consumed the pure lupine fibre-product (25 g/day) over two weeks. In study II 54 moderate hypercholesterolemic subjects consumed the same amount, incorporated in different food, over a period of 28 days.

The serum cholesterol concentration did not change in normocholesterolemic subjects. In contrast, the four-week intervention with lupine fibre-enriched food in hypercholesterolemic subjects decreased the total cholesterol by 12% ($P < 0.001$) compared to baseline. The LDL concentration was lowered by 15% ($P < 0.001$) and HDL cholesterol remained unchanged. Moreover, the lupine fibre enriched diet led to a significant decrease of the triacylglyceroles ($P=0.03$) and of the high sensitivity C-reactive protein ($P=0.02$).

Additionally, the intake of lupine fibre increased satiety and modified nutritional behaviour positively (lower intake of energy, fat, protein and cholesterol), which can support long-term weight loss and protect against diet-induced obesity.

To sum up, the results of the present studies show that lupine kernel fibre can have a positive impact on putative risk factors of atherosclerosis. The inclusion of this palatable lupine fibre into the diet can help predisposed people in prevention of coronary heart disease and the fibre-consumption can support medical therapies.

Memory Stick

INCIDENCE OF ACUTE, SUBACUTE, LATE AND VERY LATE IN-STENT THROMBOSIS IN PATIENTS WITH ACS AFTER DES AND BMS IMPLANTING

Anastasia Lucia Romano, A. Azzano, G. Zampi, F. Fini, V. Paravati, C. Gaudio

Heart and Great Vessels, Sapienza University of Rome, Rome, Italy

Introduction: Coronary stents improved the clinical outcomes of patients with ACS, but the risk of in-stent thrombosis impairs their safety.

Objective: To assess the incidence of acute, subacute, late and very-late in-stent thrombosis.

Materials and methods: January 2004 - December 2008, 1600 patients with ACS underwent a coronary study. 1300 of them were revascularized with stenting (on-label and off-label). In-stent thrombosis was detected in 24 pts who were divided into four groups according to the event's timing: A: acute (within 24 hours); B: subacute (within 1 month); C: late (within 1 year); D: very late (beyond 1 year).

Results: In-stent thrombosis occurred with an incidence of 1.8%.The acute one (9 pts:7 BMS, 2 DES) occurred with an incidence of 0.69%; the subacute one (6 pts: 2 BMS, 4 DES) of 0.46%; the late one of 0.15% (2 pts: 0 BMS, 2 DES) and the very late one of 0.51% (7 pts: 2 BMS, 5 DES).The main risk factor in the acute thrombosis was smoking (100%), associated with hypertension (100%) in the subacute one, and with hypertension and dyslipidemia (100%) in the late one, while familiarity for CAD and dyslipidemia (85.7%) prevailed in very late in-stent thrombosis.

Conclusions: Acute thrombosis occurred more frequently in pts implanted with BMS while DES entailed a greater risk of very late event. Besides, the triad dyslipidemia, smoke, hypertension prevailed in pts with acute, subacute and late thrombosis, while in the very late one, the association between familiarity for CAD, dyslipidemia and hypertension.

Memory Stick

WHY WE ARE UNABLE TO FIND CHD GENE - APOE AN EXAMPLE

Rudolf Poledne[1], J. Pitha[1], J. Hubacek[1], P. Stavek[1], R. Cifkova[2], V. Lanska[3]

[1]Laboratory for Atherosclerosis Research, [2]Dept. of Preventive Cardiology, [3]Dept. of Statistics, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Objective: After two decades of analysis of genetic background of CHD, numerous candidate genes have been identified but often not confirmed in other populations and a low relative risk of disadvantageous genotypes.

Methods: 1069 men with acute coronary syndrome (ACS) hospitalized in five coronary care units in Prague under age 65 were enrolled in 2006-2009. They were compared to age-matched individuals from 1% representative population sample of the Czech post-MONICA study.

Results: Apoprotein E genotypes containing E4 alele were significantly more frequent in ACS patients compared to controls with relative risk 1.47 (95 % CI 1.19-1.82). No significant difference of LDL cholesterol concentration was found compared to controls with significantly lower HDL cholesterol. In subgroup analysis with subsequent exclusion of smokers (n=515), diabetics (n=277) and individuals with hypertension (n=148) the odds ratio of disadvantage genotypes (+E4) increases gradually (from 1.48 to 1.71) with the highest frequency in the final group (non-smokers without diabetes, with normal blood pressure). The trend for this change was significant ($p < 0.05$). The final group (n=129) displayed lower HDL cholesterol and educational level compared to the rest of ACS patients. The most pronounced difference was in the category of a positive family history of CHD.

The effect of disadvantageous genotype in the apoE gene locus differs in different subgroups of ACS patients.

Conclusion: This non-homogeneity of the gene manifestation is affected due to environmental confounding parameters, smoking, diabetes and hypertension. This might be additional reason for the low level of reproducibility of genetic analysis in different populations.

Memory Stick

ECCENTRIC ENDURANCE EXERCISE SIGNIFICANTLY IMPROVES FASTING GLUCOSE AND GLUCOSE TOLERANCE IN NON-DIABETIC SUBJECTS

Alexander Vonbank[1,2,3], C.H. Saely[1,2,3], P. Rein[1,2,3], S. Aczel[1,2], S. Beer[1,2,3], V. Kiene[1], T. Bochdansky[2,4], H. Drexel[1,2,3,5]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Department of Physical Medicine, Rankweil State Hospital, Rankweil, Austria, [5]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Eccentric exercise (i.e. active resistance to muscle stretching, e.g. with hiking downwards) is less strenuous than concentric exercise; its metabolic effects are largely unknown. We aimed at investigating whether eccentric endurance exercise improves glucose tolerance in healthy subjects without diabetes.

Methods: We conducted a controlled eccentric exercise intervention over a training period of 8 weeks. A total of 93 non-diabetic sedentary subjects were allocated to the intervention group, performing 3 to 5 times per week eccentric endurance exercise by hiking downhill a path in the Austrian alps covering a difference in altitude of 540 meters; for the upward way a cable car was used, where compliance was recorded electronically. The control group included 25 subjects who remained sedentary. An oral glucose tolerance test (OGTT) was obtained at baseline and after 8 weeks of eccentric exercise.

Results: Fasting plasma glucose decreased significantly in the intervention group (from 97 ± 15 at baseline to 94 ± 9 mg/dl after 8 weeks of eccentric endurance exercise; $p= 0.025$), but not in the control group ($p = 0.265$). Further, glucose tolerance (quantified as the incremental area under the glucose curve) was significantly improved in the intervention group (by 8.1 %; $p < 0.001$), but not in the control group ($p = 0.231$).

Conclusion: We conclude that eccentric endurance exercise even in non-diabetic individuals significantly improves fasting glucose and glucose tolerance. Because elevated fasting and postchallenge glucose values indicate an increased diabetes risk, eccentric endurance exercise may help to prevent diabetes.

Memory Stick

A RANDOMIZED OPEN TRIAL OF VARENICLINE VERSUS NICOTINE PATCH IN ADULT SMOKERS: EFFICACY, SAFETY AND WITHDRAWAL SYMPTOMS (THE VN-SEESAW STUDY)

H. Tsukahara, K. Noda, Y. Uehara, **Keijiro Saku**

Cardiology, Fukuoka University School of Medicine, Fukuoka, Japan

We conducted a randomized controlled trial of varenicline versus nicotine patch in adult smokers, and compared efficacy, safety and withdrawal symptoms. Thirty-two adult smokers were randomly divided into a varenicline group (VG, n=16, varenicline, 0.5-2mg daily) and a nicotine patch group (NG, n=16, 52.5-7mg nicotine daily) for 12 weeks and 8 weeks, respectively. The primary endpoints were the 12- and 24-week smoking-abstinence rates, withdrawal symptoms and safety. No significant difference in abstinence rates was observed between the two groups over weeks 9-12, and weeks 9-24. The total withdrawal symptom score in VG was higher than that in NG at 2 weeks (14.8±3.5 vs. 12.2±2.4, p=0.083): in detail, the frequencies of an inability to concentrate at 2, 4, and 8 weeks (P=0.034, 0.070, 0.10, respectively), wakeful nights at 2 weeks (p=0.003), and a lack of self-composure at 4 weeks (p=0.146) were higher in VG than in NG. Adverse side effects associated with a gastro-intestinal disorder were observed in 14 cases and 1 case, and skin allergy was seen in 0 and 9 cases, respectively. BW and BMI were increased after 12 weeks in both groups, while systolic blood pressure tended to decrease in NP and LDL-C and HDL-C did not change after 12 weeks. Urinary 8-OHdG and 8-isoprostane decreased in NG group. We concluded that the selection of treatment should depend on the patient´s request based on the desired acuteness of cessation of smoking, gastro-intestinal problems, and skin allergy.

Memory Stick

IMPAIRED GLUCOSE TOLERANCE, INFLAMMATION AND MALNUTRITION SYNDROME IN OLDER PATIENTS WITH A SUBCLINICAL FORM OF PRIMARY HYPOTHYREOIDISM

Ljiljana Majnaric[1], B. Vitale[2]

[1]University "J.J. Strossmayer" Osijek, Health Center Osijek, Croatia, Osijek, [2]Ruđer Bošković Institute Zagreb, Zagreb, Croatia

Background and aims: Primary hypothyreoidism (PH) is known disease which may add to the cardiovascular (CV) risk profile. We investigated whether a subclinical form of PH, more frequently distributed in population than a clinically developed disease, can also be detrimental to the CV disease development.

Methods: Increased TSH hormone levels and situations with TSH hormone values within the upper half and fT4 hormone values within the lower half of the reference range were used as criteria for definition of a subclinical form of PH. We tested 70 patients, aged 55-74 years (median 68), without diabetes mellitus, on fT4 and TSH hormone levels and divided them in two groups according to these criteria. Differences in many clinical parameters were than tested between these two groups of patients, including: BMI, weist/hipp ratio, cognitive impairment, anxiety/depression, OGTT, HbA_{1c}, inzulin, lipids, serum total proteins, acute phase proteins, folic acid, vitamin B_{12}, homocystein, prolactin and kortizol *(median and 25%-75% range and Mann*-Whitney U test, $p< 0,05$).

Results: We found a high proportion of 25,7% subjects with a subclinical form of PH. They were characterized with lower inzulin levels,14,95 (13,10-21,80) vs.19,30 (15,90-26,55) mIU/ml, p=0,039, higher OGTT 2h glucose levels, 6,30 (5,40-7,10) vs. 5,20 (4,10-6,30) mmol/L, p=0,043, decreased serum albumin, 45,10 (41,70-46,50) vs. 46,80 (44,80-48,15) g/L, p=0,042, decreased vitamin B_{12}, 222,0 (198,0-297,0) vs. 237,0 (194,0-319,5), p=0,048, and increased kortizol, 404, 65 (335,90-457,60) vs.337,50 (253,90-415,35),p=0,038, in comparison with the controls.

Conclusions: Impaired glucose tolerance, inflammation, malnutrition and neuroendocrine stress axis hyperactivity syndrome characterizes a subclinical form of PH.

Memory Stick

IMPORTANCE OF INSULIN RESISTANCE AND GLUCOSE INTOLERANCE IN PREMENOPAUSAL WOMEN WITH POLYCYSTIC OVARIES AND POLYCYSTIC OVARIAN SYNDROME (PCOS)

Daniela Paul[1], I. Pantea[1,2], L. Nedelcu[1,2], A. Stoicescu[3,4], C. Scarneciu[1,2]

[1]Internal Medicine, County Emergency Hospital of Brasov, [2]Internal Medicine, [3]Endocrinology, Faculty of Medicine, University of 'Transilvania', [4]Endocrinology, County Emergency Hospital of Brasov, Brasov, Romania

Objective: The aim of this study was to assess whether insulin resistance and glucose intolerance in women with polycystic ovaries and PCOS contribute to an additional risk for metabolic syndrome and coronary artery disease.

Methods: The study included 78 premenopausal women (aged 20-48 years old) diagnosed with polycystic ovaries and/or PCOS. All patients underwent complex clinical examination including anthropometric measurements (BMI, waist circumference) and lower abdominal echography. Biological tests were done: TSH, prolactin levels, 17-hydroxiprogesterone,progesterone levels, DHEAS, LH, FSH, impared glucose, OGTT, insulin, lipid profile. We also considered lifestyle risk factors like smoking.

Results: The lot was divided into 2 groups. Group 1 consisted of 36 patients that presented either glucose intolerance, insulin resistance or diabetes from whom 19(52.78%) patients were obese (IMC>30, waist circumference>0.85) and 17(47.22%) were overweight (IMC-25-30, waist circumference>0.80). First group consisted of 26(72.22%) smokers. Group 2 consisted of 42 women without insulin resistance from whom 18(42.85%) were obese (IMC>30, waist circumference>0.85), 11(26.2%) were overweight (IMC-25-30, waist circumference>0.80) and 13(30.95%) patients had normal weight. The second group presented 18(42.85%) smokers. We also studied the prevalence of metabolic symptoms for polycystic ovary and PCOS: chronic anovulation without hirsutism (38.46% cases), amenorrhea with normal prolactin level (29.48% cases), hirsutism with regular cycles (10.25% cases) and polycystic ovaries (21.79% cases).

Conclusion: Most women with PCOS are obese or overweight and developed insulin resistance and glucose intolerance. Obesity per se is a cause of insulin Syndrome X. Moreover, smoking seems to contribute to insulin resistance and glucose intolerance mechanisms. Therefore, measures to decrease this condition may have to be considered earlier to decrease the potential risks of developing diabetes mellitus and coronary artery disease at later ages of life in both overweight and normal weight women who have PCOS.

Memory Stick

URIC ACID, ENDOTHELIAL FUNCTION AND CARDIOVASCULAR RISK

E.E. Babes, **Victor Vlad Babes**, A. Ardelean, M.I. Popescu

Faculty of Medicine, Oradea, Romania

Background: Previous studies have raised the possibility that UA might be an independent, causal risk factor for cardiovascular disease.

The aim of the study was to evaluate the relationship between UA and endothelial function in patients with hypertension.

Methods: A total of 106 newly diagnosed never treated patients with uncomplicated essential hypertension (56 men, mean age 49, 6 ±6,8y) were studied. Classical risk factors, UA, creatinine and C reactive protein (CRP) were determined. Endothelial function was estimated by flow-mediated dilatation (FMD) of the brachial artery. Patients were distributed in two groups: high UA ≥ 5 mg/dl (n=49) and low UA < 5 mg/dl (n=57).

Results: Patients with high UA were older (p=0,002), had higher systolic blood pressure (SBP) (p< 0,001), higher creatinine (p< 0,01) and higher CRP (p=0, 05). There were no differences between groups regarding BMI, lipid profile, diastolic BP, percentage of smokers. FMD was markedly lower in patients with high UA (5,77 ± 1,55%) vs (7,57 ± 1,23%) (p< 0, 0001). In multiple regression analysis independent predictors for FMD were: creatinine (r= - 0, 67, p< 0, 0001), serum UA - second correlate of FMD (r= - 0, 64, p=0, 0002) and SBP (r= - 0, 56, p=0, 0015) (R^2=0,73).

Conclusions: Hyperuricemia in patients with essential hypertension is associated with endothelial dysfunction. This association is independent of classical risk factors and CRP and may contribute to cardiovascular morbidity. Serum UA represents a possible new target for the reduction of morbidity and mortality associated with hypertension and cardiovascular disease.

Memory Stick

NEW EVENT OF MYOCARDIAL INFARCTION (MI) IN PATIENTS WITH PAST MI: AGE, SEX AND TREATMENT DIFFERENCES

Antonios Ioannidis, K. Oikonomou, M. Ntalapascha, E. Karapatsoudi, I. Karantoumanis, K. Karali, P. Makridis

Department of Cardiology, General State Hospital of Edessa, Edessa, Greece

Background: Limited data exists concernimg the pre- and post- admission medications of patients with history of MI suffering from a new event of MI.

Aim: We studied the differences between the sexes regarding the pre- and post- admission medication, the type of in-hospital treatment, the type of MI, along with routine clinical and laboratory features.

Materials and methods: 315 patients admitted to the hospital for MI betweeen 1995 and 2009 were retrospectively enrolled.

Results: Data, collected from patients archives, of 245 men and 70 women were incorporated, with mean age of 69±10 years and 75±7,9 years respectively ($p< 0.05$). 21,2% of men received thrombolysis in contrast to only 8,6% of women (p:0.016). Pre-admission medication showed no differences, except for a trend of reduced prescription of aspirin in women (47,1% vs 60%, p:0.055). An overall increase in the prescription of medications (pre- vs post admission) as suggested by the recent guidelines was noticed (aspirin, clopidogrel, β-blockers, ACE-inhibitors, statins) ($p< 0.05$). No statistical significance was observed in relation to ejection fraction, systolic and diastolic blood pressure, cholesterol, renal function, haemoglobulin and the type of MI.

Conclusions: We concluded that women compared to men suffered from a new event of MI in more advanced age, they were less commonly pre-admission prescribed aspirin, and received thrombolysis in fewer cases. We confirmed an icremental adherence to international guidelines about prescribed medications regarding secondary prevention after MI.

Memory Stick

ASSOCIATIONS BETWEEN SERUM ALANINE AMINOTRANSFERASE LEVELS AND CARDIOVASCULAR RISK FACTORS IN JAPANESE MEN

Yukio Ikeda[1], Y. Kumon[2], T. Ohguro[3], N. Hisakawa[1], A. Ishibashi[4], H. Takata[4], M. Inoue[4], Y. Terada[4]

[1]Diabetes Center, Kochi Memorial Hospital, Kochi, [2]Department of Laboratory Medicine, Kochi Medical School, Nankoku, [3]Department of Health Examination, Kochi Red Cross Hospital, Kochi, [4]Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Nankoku, Japan

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is now regarded as the hepatic component of the metabolic syndrome. Serum alanine aminotransferase (ALT) is closely related to liver fat accumulation and is widely used as a biomarker for NAFLD. We examined whether the serum ALT level is associated with insulin resistance, adipocytokine and fetuin-A levels, and other cardiovascular risk factors in Japanese men.

Methods: We recruited 295 unrelated Japanese men, without known chronic disease including diabetes mellitus or history of regular drug use, and who were negative for hepatitis B or C (age, 52 ± 11 years). Based on a 75-g oral glucose tolerance test, 192 subjects had normal glucose tolerance (NGT), 88 had impaired glucose tolerance and/or impaired fasting glucose (impaired glucose regulation, IGR) and 15 had diabetes mellitus.

Results: No differences in ALT level were detected between subjects with NGT, IGR and diabetes mellitus. ALT levels were significantly correlated with age, BMI, waist circumference, blood pressure, triglycerides, fasting plasma glucose, 2-h plasma glucose, high-sensitive CRP ($r=0.137$, $P< 0.05$), HOMA-insulin resistance index ($r=0.474$, $P< 0.001$), adiponectin ($r=-0.275$, $P< 0.001$), and leptin levels ($r=0.241$, $P< 0.001$). ALT levels were weakly but not significantly correlated with fetuin-A levels ($r=0.101$, $P=0.084$). Multivariate analysis showed that high HOMA-insulin resistance index, young age, high BMI, high 2-h plasma glucose, high CRP and high leptin were significantly associated with elevated ALT levels.

Conclusions: Our data suggest that elevated serum ALT is an independent biomarker for visceral adiposity and impaired insulin sensitivity in Japanese men.

Memory Stick

OBESITY AND CARDIOVASCULAR RISK IN NORTHERN GREECE. ATHOS CARDIO GREECE STUDY

Apostolos Efthimiadis, I. Kanonidis, G. Sakantamis, C. Manes, E. Paschalidou, I. Efthimiadis, S. Pavlidou, A. Tavridou

Northern Greece Atherosclerosis Society, Thessaloniki, Greece

Objective: To study the prevalence of obesity and its relation to cardiovascular risk in a Northern Greek population sample.

Method: 3000 subjects (54% males and 46% females) with mean age 62±13 years from Northern Greece were examined during an epidemiological project the last three years. All subjects gave informed consent form and completed a questionnaire on personal and family medical history. Demographic/anthropometric characteristics and blood pressure were recorded. Biochemical parameters (total cholesterol, HDL, LDL, triglycerides) were measured by the Cholestech Kit (dry chemistry method). According to BMI, all subjects were divided into three groups.

Group A: Normal (BMI ≤ 25 kg/m²).

Group B: overweight (25 < BMI < 30 kg/m²) and

Group C: obese (BMI ≥ 30 kg/m²). SPSS 15.0 was used for the epidemiological analysis.

Data are presented as mean±standard deviation.

Results: From the 3000 subjects, 29.4% found to be normal, 41.7% overweight and 28.9% obese. 73% of all subjects were smokers (over a package/day). 50.5% of the overweight and 50.2% of the obese group had dyslipidaemia. Hypertension also had a higher prevalence over the overweight and obese group. Atheromatic index was higher over the obese group (4.7±1.6) followed also by a higher risk score (27%).

Conclusions: Despite the Mediterranean diet, obesity in Greece has a high prevalence. Most of the subjects studied, had more than one risk factor something that increased the overall cardiovascular risk score. Lipid profile and hypertension found to be strongly related to BMI and waist circumference.

Memory Stick

ADELINA: EFFECTS OF HIGH-DOSE ATORVASTATIN ON ESTIMATED CARDIOVASCULAR RISK AND ADIPOKINE PRODUCTION IN DYSLIPAEMIC NON-DIABETIC PATIENTS WITH METABOLIC SYNDROME

Francisco Martinez-Martin[1], P. Soriano Perera[2], The ADELINA Study Group

[1]*Endocrinology and Nutrition - Hypertension Clinic, Universitary Hospital Dr. Negrin, Las Palmas de Gran Canaria,*
[2]*Endocrinology and Nutrition, Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain*

Objective: To assess the reduction of estimated cardiovascular risk and the changes in adipokines with high-dose atorvastatin treatment in hypercholesterolemic patients with metabolic syndrome.

Methods: 102 non-diabetic patients with metabolic syndrome and uncontrolled hypercholesterolemia (LDL-C 100-160 mg/dl) were enrolled for the study. Previous antihyperlipidaemic treatments were washed out for 4 weeks. All patients received atorvastatin 40 mg/day. After 14 ± 2 weeks, the dose of atorvastatin was doubled in patients who maintained LDL-C > 100 mg/dl. The study concluded after another 14 ± 2 weeks. Blood pressure, heart rate, lipid profile, CRP, adiponectin, TNF-α and PAI-I were measured in each visit. The cardiovascular risk was estimated by the local CDC adaptation of the Framingham Table.

Results: 93 patients completed the protocol, of which 39 (41.9%) had their doses doubled. The absolute cardiovascular risk was reduced (p = 0.008) from a median of 19.3% (interquartile range 12.8 - 29.3%) to 13.7% (9.6 - 21.1%). CRP and PAI-1 were reduced (by 37.3%, p= 0.002, and 19.3%, p= 0.032). Adiponectin and TNF-α were not significantly modified. Two patients (1.96%) were withdrawn due to moderate muscular pain. There was a modest reduction in blood pressure (median 6/3 mmHg, interquartile range 4/2 - 9/5 mmHg; p = 0.037).

Conclusions: Treatment with high-doses atorvastatin in non-diabetic patients with moderate hypercholesterolemia and metabolic syndrome was highly effective, and well tolerated. The reduction of the estimated cardiovascular risk was highly significant: 5.6% for absolute risk and 29.0% for relative risk. Blood pressure, CRP and PAI-1 were significantly reduced.

Memory Stick

CORONARY HEART DISEASE IN PATIENTS WITH STROKE- PATHOPHYSIOLOGIC CORRELATIONS EPIDIOMIOLIC STUDY IN THE GREEK POPULATION

Ioannis Kanonidis[1], K. Martiadou[1], G. Rompolas[1], N. Sailer[2], S. Tzotzis[2], C. Kagiadaki[1], D. Eclisiarhos[1], G. Sakantamis[3]

[1]2nd Department of Cardiology, Aristotle University of Thessaloniki, [2]1st Department of Internal Medicine, Hippokration Hospital, [3]2nd Department of Cardiology, Arhus University Hospital, Thessaloníki, Greece

Introduction: The study aims to evaluate the incidence of Coronary Heart Disease(CHD) and its possible pathophysiologic implications in patients with stroke.

Material and methods: The study included all patients hospitalized for stroke during a period of 2 years in our department. All patients had a thorough investigation on admission including CT scan, ECG, Echo(transthoracic and transesophageal)laboratory test, e.tc.

Results: 539 patients, 306 men and 233 women of mean age 74,9 years were hospitalized for stroke. 501 had a thromboembolic episode and 38 an hemorrhagic one. Of the thromboembolic group 327 (65,25%) had a transient episode (TIA) and 174 (34,5%)had a permanent sequel.

History of CHD was reported in 144 (26,76%) patients, 96 men (31,4%) and 48 women (20,4%). Hemorrhagic stroke developed 15pts and thromboembolic stroke 129pts. Of those with thromboembolic stroke 89 (69%) presented with TIA and 40 (31%) with permanent stroke. All patients had a history of acute myocardial infarction and were on antiplatelate treatment. Extrasystolic activity or ventricular arrhythmias were noticed in 39 (30%) and Atrial fibrillation in 16 (12,4%) patients. s. Clinical signes of heart failure or severe ventricular dysfunction was found in 67 patients. All patients had history of hypertension and 25 of diabetes mellitus.

Conclusion: CHD as coexisting factor is found in about one forth of stroke cases. It is mostly related to thromboembolic events.

It is more frequent in women than in men. Its involvement in the pathogenesis of stroke, it is invoked in cases complicated with heart failure and/or arrhythmias.

Memory Stick

ASSOCIATION BETWEEN LOW HDL-C AND FAMILY HISTORY OF PREMATURE CORONARY HEART DISEASE IN SPANISH STATIN-TREATED PATIENTS. THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], C. Guijarro[3], E. Alegria[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hospital Clinico Univesitario de Santiago de Compostela, Santiago de Compostela, [3]Hospital de Alcorcon, Alcorcon, [4]Clinica Universitaria de Pamplona, Pamplona, [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Introduction: Patients with a family history of coronary heart disease (CHD) have an additional risk factor of cardiovascular disease (CVD). Data suggest that low-HDL-C is also an important CV risk factor and statins have a limited action on it. In this analysis we assess the association between low-HDL-C and a family history of premature CHD (FamH-CHD) in DYSIS-Spanish patients.

Methods: Analysis of 3710 Spanish patients of DYSIS (22063 participants in Europe and Canada) treated with statins. We used the ATP-III recommendations to classify patient´s risk and define the LDL-C goal and normality or not of the HDL-C and TG concentrations.

Results: In 3710 patients, 19.9% had a FamH-CHD (56.7% men and 43.3% women). Of those, 60.4% did not achieve the LDL-C goal vs. 47.4% of patients without FamH-CHD (n=2995; p< 0.0001). The prevalence of low HDL-C was higher in these patients, 35.5% vs. 28.3% (p< 0.001), compared to patients without FamH-CHD. The prevalence of CVD in patients with a FamH-CHD was 43.9% and it was more frequent than in those without family history, 33.5% (p< 0.001).

Conclusions: In this analysis of Spanish statin-treated patients, FamH-CHD was associated with a higher CV risk and higher prevalence of low HDL-C. Most of the patients with FamH-CHD did not achieve the LDL-C goal. It may be of interest to manage the dyslipidemia as a multidimensional approach in order to reach LDL-C targets and to treat other lipid abnormalities that are frequent in these patients (low HDL-C and high TGs).

Memory Stick

IDENTIFICATION OF GENES ASSOCIATED WITH QT INTERVAL USING THE 50K CARDIO-METABOLIC SNP CHIP: RESULTS FROM THE WHITEHALL II STUDY

Sonia Shah[1], F. Drenos[2], T. Shah[3], J. Palmen[2], R. Sofat[3], M. Kumari[4], J. Pallas[5], P. MacFarlane[6], J. Whittaker[7], P. Talmud[2], S. Humphries[2], A. Hingorani[3,4]

[1]UCL Genetics Institute, [2]Division of Cardiovascular Genetics, Department of Medicine, [3]Centre for Clinical Pharmacology, [4]Department of Epidemiology and Public Health, [5]Wolfson Institute for Biomedical Research, UCL, London, [6]Division of Cardiovascular & Medical Sciences, Gardiner Institute, Glasgow, [7]Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

Background: The QT interval on an electrocardiogram is a measure of the total time from ventricular depolarization to repolarization. QT interval, a heritable quantitative trait, and its prolongation by drugs, cardiac disease and electrolyte abnormalities are associated with the risk of developing ventricular arrhythmias and sudden cardiac death.

Methods and aims: Using a dense gene-centric SNP array (50,000 SNPs in 2100 genes implicated across a range of cardiovascular, metabolic and inflammatory traits) we analysed data from 5059 Caucasian individuals from a large prospective observational study of middle aged civil servants (Whitehall II) in which ECG QT interval was available from digitised ECG data.

Results: We observed multiple associations at $P < 1 \times 10^{-4}$ of SNPs in *NOS1AP*, *KCNH2* and *PLN*. *NOS1AP* codes for an adaptor protein known to be involved in myocardial repolarisation. *KCNH2* codes for a potassium ion channel protein and mutations in the gene alters the activity of the channel leading to the abnormal heart rhythm characteristic of short QT syndrome. *PLN* encodes cardiac phosphlamban which has been linked to dilated cardiomyopathy and heart failure in mouse models. Additional novel loci identified will require replication in separate population studies and further analysis will be done to refine the genetic associations to identify independent signals of association.

Conclusions: Common variants in the genes *NOS1AP*, *KCNH2* and *PLN* influence QT interval in healthy men and women, confirming results from recent whole genome analyses.

Memory Stick

IL6 AND *IL10* GENES ARE ASSOCIATED WITH UNFAVOURABLE OUTCOMES IN PATIENTS WITH ACUTE CORONARY SYNDROME

Konstantin Blagodatskikh[1], J. Agapkina[1], A. Nikitin[1], M. Evdokimova[2], V. Osmolovskaya[2], V. Nosikov[1], D. Zateyshchikov[2]

[1]Molecular Biology, National Research Centre "GosNII Genetika', [2]Russian President's Educational Scientific Medical Centre, Moscow, Russia

Objectives: It is obvious that individual genetic features play important role in the unfavourable outcomes (UO) after acute episodes of CAD. We suggested that the *IL6* and *IL10* genes which included in inflammation processes could play an important role in the forming of genetic predisposition to development of UO.

Methods: 1145 patients (717 male, 428 female), aged 61.6±0.35 years with acute coronary syndrome were studied. 933 patients had arterial hypertension, 367 - a previous ischemic stroke. Allele identification was performed using RFLP method.

Results: We have found association of UO with *G(-174)C* polymorphism of *IL6* gene and *G(-1082)A* of *IL10* gene. In case of *G(-174)C* polymorphism of *IL6* gene we have found that the carriers of *GG* genotype ($\chi 2$=5.2, p=0.023) have the lower survival time (2.10±0.08 years), whereas the carriers of *CG* and *GG* genotypes have the higher survival time (2.33±0.06 years). In case of *G(-1082)A* polymorphism of *IL10* gene we have found that the carriers of *AA* and *AG* genotypes ($\chi 2$=5.0, p=0.026) have the lower survival time (2.10±0.07 years), whereas the carriers of *GG* genotype have the higher survival time (2.32±0.06 years).

Conclusion: The result of our study are evidence that the carriers of *GG* genotype of *G(-174)C* polymorphism of *IL6* gene and the carriers of *AA* and *AG* genotypes of *G(-1082)A* polymorphism of *IL10* have the higher risk of UO in patients with acute coronary syndrome.

Funding: A research grants from CJSC Taatta Bank and Russian Foundation for Basic Research.

Memory Stick

ASSOCIATION OF THE CHOLESTEROL ESTER TRANSFER PROTEIN GENE (*CETP)* WITH CORONARY ARTERY DISEASE

Alexey Brovkin[1], E. Dankovtseva[2], A. Nikitin[1], L. Minushkina[2], D. Zateyshchikov[2], V. Nosikov[1]

[1]National Research Centre 'GosNII Genetika', [2]Russian President's Educational Scientific Medical Centre, Moscow, Russia

Background and aims: In the current investigation, we have studied an association with coronary artery disease (CAD) of candidate gene, which product is involved in regulation of lipid transport: cholesterol ester transfer protein (*CETP*).

Patients and methods: Two groups of Russian patients living in Moscow have been formed: group with acute unstable angina (n = 293) and control group (n = 132) in which all patients had no clinical CAD. Allele identification was performed using PCR, restriction endonuclease cleavage and PAAG separation.

Results: We have not found any association with CAD of polymorphic markers *TaqIB, C(-724)T* and *A(-629)C* of *CETP* gene. But in case of polymorphic marker *Ile405Val* of *CETP* gene we have found that the carriers of *Ile/Ile* (OR = 0.44, p = 0.0006; CI = 0.27 - 0.73) have the lower risk of coronary artery disease, whereas the carriers of *Ile/Val* (OR = 2.67, p = 0.0006; CI = 1.61 - 4.42) have the higher risk of coronary artery disease.

Conclusion: The result of our study is evidence that the carriers of *Ile/Val* genotype of *Ile405Val* polymorphism of *CETP* gene have the higher risk of coronary artery disease.

Memory Stick

ANTI-PHOSHOLIPID ANTIBODIES IN ACUTE CORONARY ATHEROTHROMBOSIS

Ivana Burazor[1], A. Vojdani[2], M. Burazor[1]

[1]Cardiology, University Clinical Center in Nis, Nis, Serbia, [2]Immnosciences Lab, Inc, Los Angeles, CA, USA

The combined presents of anti-phospholipid antibodies and thrombosis is recognized as the antiphospholipid syndrome (APS). Also, atherosclerosis is a histologic process in which the immune system has important role. Based on this data and our experience we aimed to investigate the possible role of anti-phospholipid antibodies in acute coronary atherothrombosis.

Methods and results: The study included 312 participants; of whom 112 were patients with ACS (60.74 ±4.97 years of age, majority were males) and 200 were age and sex matched controls with no known coronary artery disease or other pro-coagulant state (lupus, deep vein thrombosis etc). Patients with previous infection, surgery,cancer, trauma or concomitant reumathological diseases were excluded from the study. Blood was sampled, frozen and sent on dry ice to Immunosciences Lab. Inc (USA) for analyzes. All traditional risk factors were noted.

Several antibodies of antiphospholipid syndrome were determined: anti-prothrombin (aPL), anti-cardiolipin (aCL) and anti- beta 2 glycoprotein 1. In addition, anti-coagulant protein C was determined. Our data showed significant prevalence of examined antibodies in patients with ACS. The levels of circulating antibodies were significantly higher in patients ($p< 0.001$). Protein C was 144.57±36% in patients and 101.30±23.11% ion controls, $p< 0.001$.There was a linear correlation between anti-phospoholipid antibodies themselves and between protein C and aCL ($p< 0.01$). Linear regression confirmed the involment of APS in acute coronary atherothombosis.

In conclusion our data conifirmed that antiphospholipid antibodies are presents in acute coronary atherothrombosis. APS might represent a state which induce strong immune response in coronary arteries.

Memory Stick

ACUTE CHANGES IN SERUM HEAT SHOCK PROTEIN 27 ANTIGEN AND IGM CONCENTRATIONS IN PATIENTS WITH ACUTE CORONARY SYNDROME

Dayangku Fatiha Pengiran Burut[1], S. Shafi[1], G. Ferns[1,2]

[1]Faculty of Health and Medical Sciences, University of Surrey, Guildford, [2]Institute of Science in Technology and Medicine, University of Keele, Stoke, UK

Background: Heat shock protein 27 (Hsp27) is expressed in various muscle tissues especially myocardial tissue in both the vascular and cardiac compartments. During an acute coronary event, Hsp27 may be released into the circulation leading to an immune response being directed against this protein.

Objective: To investigate the change in serum Hsp27 antigen and its immunoglobulin M (IgM) antibody in patients with chest pain, at admission (< 12 hours since the episode of chest pain), and 12 hours after admission.

Method: Controls (n=37) were staff and students of the University of Surrey and the Royal Surrey County Hospital (RSCH).

Patients (n=74) were from the RSCH admitted with chest pain and subdivided into troponin -ve (Trop-) and troponin +ve (Trop+) groups. Hsp27 antigen and anti-IgM antibody were measured by enzyme-linked immunosorbent assays developed inhouse.

Results: On admission, serum Hsp27 was significantly lower in controls compared to Trop- ($p< 0.001$) and Trop+ ($p< 0.001$) patients. For Trop+ patients only, serum Hsp27 concentration for the 12 hour sample was significantly lower compared to the sample on admission ($p=0.04$) and so were the IgM antibody titres ($p=0.03$).

Conclusion: The presence of higher serum Hsp27 for both patient groups with chest pain may indicate that Hsp27 is released into the circulation in the absence of infarction. The anti-Hsp27 IgM antibodies function to clear this protein from the circulation causing the drop in both protein and antibody levels 12 hours post admission in the patients with a myocardial infarction.

Memory Stick

INTIMA-MEDIA THICKNESS, BODY MASS INDEX AND GLUCOSE METABOLISM IN ADOLESCENT GIRLS AND YOUNG WOMEN

Antonina Starodubova[1], O. Kisliak[1], D. Sargaeva[1], G. Storozhakov[1], V. Sergienko[2], V. Govorun[2], A. Kopelev[1]

[1]Russian State Medical University, [2]Research Institute of Physicochemical Medicine, Moscow, Russia

Objective: The aim of the study was to investigate the association between common carotid artery intima-media thickness (CCA IMT), an early marker of atherosclerosis, body mass index (BMI) and glucose metabolism in adolescent girls and young women.

Methods: 60 young females 14-25 y.o. (18,50±3,54) with obesity (BMI 35,20±4,78 kg/m^2) and 15 age-matched nonobese healthy young females (20.29±2.16 kg/m^2) were enrolled. CCA IMT was assessed by B-mode ultrasound. The anthropometric measurements, oral glucose tolerance tests with insulin and C-peptide were performed. The level of insulin resistance was estimated using the homeostasis model assessment (HOMA-IR).

Results: Patients with obesity had a markedly greater carotid IMT (0,52±0,08 vs 0,41±0,03 mm; P < 0.001) than control subjects. Univariate analysis showed a significant positive correlation between CCA IMT and BMI (R=0,49), fasting levels of glucose (R=0,31), insulin (R=0,39) and C-peptide (R=0,34), HOMA-IR (R=0,38) (P< 0,01). No correlation was obtained between CCA IMT and smoking, family history of type 2 diabetes or early cardiovascular disease, visceral obesity, or the lipid profile. Multiple regression analysis showed that when controlled for age, and BMI, only HOMA-IR (beta =0,308) and fasting glucose (beta =0,278) remained an independent determinant of CCA IMT (p< 0,05). In patients with obesity CCA IMT was associated only with fasting glucose levels (R=0,31, p< 0,01).

Conclusions: These results support the importance of obesity and glucose metabolism disorders as major risk factors for atherosclerosis in young females. Fasting hyperglycemia may accelerate the development of atherosclerosis and increase the risk for cardiovascular disease in young obese females.

Memory Stick

PREVALENCE OF ABDOMINAL OBESITY AND ITS ASSOCIATION WITH BLOOD PRESSURE

Gábor Simonyi[1], L. Halmy[2], T. Csatay[3], M. Medvegy[4], R.J. Bedros[1]

[1]5th Department of Internal Medicine and Cardiometabolic Center, Flor Ferenc Hospital of County Pest, Kistarcsa, [2]Platon Health Center, [3]Police Health Center, Budapest, [4]Cardiology, Cardiometabolic Center, Flor Ferenc Hospital of County Pest, Kistarcsa, Hungary

Introduction: Abdominal fat deposition (AFD) is well defined and an important risk factor for cardiovascular diseases. Waist circumference (WC) is an easy way of measuring AFD. IDF WC cut-points of ≥94 cm and ≥80 cm for men and women.

Aims: To determinate the proportion of AFD and its association with blood pressure parameters.

Patients and methods: We studied 20499 Hungarian policemen 18543 men, age 18-61 y. We measured blood pressure (BP), pulse pressure (PP), mean arterial pressure (MAP), body mass index (BMI) and WC.

Results: We have found 9492 subjects with AFD (46.3%). The distribution of AFD by gender was: 46.53% men and 49.2% women of the total subjects. Systolic BP was 135.21±16.00 mmHg, diastolic BP was 84.12±10.53 mmHg, MAP was 101.15±11.32 mmHg and PP was 51.08±11.76 mmHg in men with AFD. Systolic BP was 127.33±15.86 mmHg and diastolic BP was 79.81±10.11 mmHg MAP was 95.64±11.03 mmHg and PP was 47.53±11.69 mmHg in women with AFD. We have found significant difference in patients with AFD and with no AFD in both gender in systolic and diastolic BP ($p< 0.0001$), PP ($p< 0.0001$), MAP ($p< 0.0001$) and heart rate ($p< 0.0001$). The BMI ($p< 0.0001$), waist ($p< 0.0001$) and hip ($p< 0.0001$) circumference and W/H ratio ($p< 0.0001$) correlated significantly in both gender with systolic, diastolic BP, MAP, PP and heart rate.

Conclusions: Our findings showed significant prevalence of abdominal fat deposition in this “normal” population. Lifestyle modification, body weight reduction is recommended for these patients.

Memory Stick

RELATIONSHIP BETWEEN PLASMA ADMA AND HOMOCYSTEINE LEVELS AND CORONARY ARTERY DISEASE

Salim Isbilir

Beytepe Military Hospital, Ankara, Turkey

Homocysteine and ADMA are emerging risk factors for atherosclerosis. We examined the levels of plasma total homocysteine (tHcy) and ADMA in patients with angiographically documented coronary artery disease (CAD) with or without a history of MI and in age-matched controls and the relation between their plasma levels and the degree of atherosclerosis. 80 consecutive patients who were undergoing coronary angiography at the Cardiology Department were questioned about CAD risk factors.

According to the coronary angiography results 40 patients who were diagnosed as CAD formed the case group and 40 patients with entirely normal coronary arteries formed the control group. The degree of stenosis in each coronary segment was scored with 0 point for ≤25% stenosis, 1 point for 26-50% reduction, 2 points for 51-75% reduction, 3 points for 76-90% reduction in vessel diameter, and 4 points for >90% stenosis. Number of patients with hypertension differed significantly between groups ($p< 0.05$). Both plasma tHcy (16,69±5,31 µmol/L versus 12,01±4,91µmol/L, $p< 0.01$) and plasma ADMA (2,10±0,70 µmol/L versus 1,17±0,50µmol/L, $p< 0.01$) levels were significantly higher in CAD patients than controls. No significant relation between plasma tHcy and classical risk factors was found for cardiovascular disease. ADMA levels did not show any relation with classical risk factors except hypertension (pearson $r=0.275$, $p=0.014$). Plasma ADMA levels were significantly higher in patients with hypertension (1.877 µmol/L versus 1.455 µmol/L $n=80$, $p=0.014$). Both plasma ADMA (spearman $r=0.439$, $p=0.001$, $n=40$) and plasma tHcy (spearman $r=0.423$, $p=0.001$, $n=40$) levels showed significant positive correlation with coronary atherosclerotic score.

Memory Stick

INFLUENCE OF VITAMIN STATUS AND GENETIC POLYMORPHISM ON PLASMA HOMOCYSTEINE LEVEL IN RUSSIAN PATIENTS WITH STABLE CAD

Olga Shakhmatova[1], A. Komarov[1], D. Rebrikov[2], I. Kofiadi[2], T. Kotkina[1], E. Panchenko[1]

[1]Russian Cardiology Research and Production Center, [2]DNA-Technology, Moscow, Russia

Plasma homocysteine (Hcy) level have been shown to be an important risk factor for CAD. Five polymorphisms (MTHFRC677T, MTHFRA1298C, MTRA2756G, MTRRA66G, TCNC776G) and some clinical factors (reduced folat and cobalamin level, diabetes mellitus (DM), renal insufficiency and atherosclerosis severity) may contribute to hyperhomocysteinemia.

Purpose: To investigate determinants of hyperhomocysteinemia in Russian patients with stable CAD.

Methods: 506 pts (388 male, mean age 59.4±12.2 years) with stable CAD were enrolled in the study. Renal function was estimated by creatinine clearance (CrCl), Cocroft-Gault formula. Atherosclerosis severity was evaluated by concomitant CVD and/or PAOD. Hcy, folat and cobalamin plasma concentrations were measured. Polymorphisms were detected based on the real-time PCR.

Results: Mean Hcy was 14.3±4.6µmol/l. 432 (85.4%) pts had hyperhomocysteinemia (Hcy>10µmol/l). According to univariate analysis, Hcy level was related to folat plasma level (Spearman rank -0.23, $p< 0.0001$), B12 plasma level (Spearman rank -0.24, $p< 0.001$) and CrCl < 90 ml/min (Hcy level 15.2µmol/l vs 13.5µmol/l, $p< 0.02$). According to ANOVA with continuous covariances (using the nested models), folat plasma level (β-coefficient -3.86, $p< 0.0001$), cobalamin plasma level (β-coefficient -5.73, $p< 0.0001$) and MTRR 66AA genotype (β-coefficient 10.71, $p< 0.0005$) were the only three independent predictors of hyperhomocysteinemia. Also Hcy level was related to combined condition - TCN 776G mutation with account taken of low cobalamin plasma level (β-coefficient -0.03, $p< 0.0001$).

Conclusion: In Russian pts with stable CAD Hcy level is related to plasma concentration of folat and cobalamin, MTRR66AA genotype and TCN 776G mutation with account taken of low cobalamin plasma level.

Memory Stick

AGE, SEX AND SOCIOECONOMIC STATUS LARGELY DETERMINE SMOKING-RISK AWARENESS

Theodoros Chimonas[1], P. Petrikos[2], E. Kostopoulos[2], E. Fanouraki[3], D. Panagiotakos[4], E. Chimonas[3], M. Elisaf[1]

[1]University of Ioannina, Ioannina, [2]251 Hellenic Airforce General Hospital, [3]Hellenic Atherosclerosis Society, [4]Harokopio University, Athens, Greece

Purpose: One of the most important risk factors for smoking is the general population's awareness of the dangers of tobacco. Therefore, we sought to explore the level of knowledge regarding tobacco-related health risks among a Greek population.

Methods: The study sample consisted of 2547 unselected adults, aged 18 to 65 (mean age 29.1±8.3 years old), 90.9% male and 9.1% female, belonging to Hellenic Air Force personnel, who filled out an anonymous questionnaire. The questions asked included whether smoking is harmful, whether it can cause lung disease, cancer, heart disease, impotence, premature death, strokes or any of the above, whether it is addictive and whether passive smoking is harmful.

Results: Among the participants, 49.0% were smokers. No difference was observed between smokers and non-smokers regarding age, sex, years of academic training or family income. Based on the answers to the above questions, a "knowledge index" was created regarding smoking. Every participant could achieve a score from 0 to 10. The average score was 7.4±2.1. A significantly higher score was achieved by non-smokers vs smokers (7.7 vs 7.2, $p< 0.001$) and women vs men (7.9 vs 7.4, $p< 0.01$). Furthermore, the knowledge index showed a positive correlation to the participants' age, years of academic training and family income ($p< 0.01$).

Conclusions: While almost everyone agreed that smoking is harmful for one's health, many people were not familiar with the severity of this risk. Older, more educated and financially better off individuals gave more correct answers regarding the dangers of tobacco use.

Memory Stick

ACCEPTANCE OF MEASURES TO CURTAIL TOBACCO USE AMONG THE GREEK POPULATION

Theodoros Chimonas[1], P. Petrikos[2], P. Papasavvas[2], C. Karkadaris[3], D. Panagiotakos[4], E. Chimonas[5], M. Elisaf[1]

[1]University of Ioannina, Ioannina, [2]251 Hellenic Airforce General Hospital, [3]Hellenic Airforce Medical Centre, [4]Harokopio University, [5]Hellenic Atherosclerosis Society, Athens, Greece

Purpose: The success of any measures undertaken to curtail tobacco use depends largely on their acceptance by the general population. Therefore, we sought to examine the level of acceptance of various possible measures against smoking among a Greek population.

Methods: The study sample consists of 2547 unselected adults, aged 18 to 65 (mean age 29.1±8.3 years old), 90.9% male and 9.1% female, belonging to Hellenic Air Force personnel, who filled out an anonymous questionnaire.

Results: Among the participants, 49.0% were smokers. Against all measures to curtail tobacco use were 2.7% of non-smokers vs 13.4% of smokers ($p< 0.001$). 83.5% of non-smokers vs 58.4% of smokers agree with smoking being banned in all public areas ($p< 0.001$). 45.8% of non-smokers vs 28.5% of smokers agree with increasing the price of cigarettes ($p< 0.001$). 54.8% of non-smokers vs 47.0% of smokers agree with increasing the minimum required age for the purchase of cigarettes ($p< 0.001$). 70.7% of non-smokers vs 51.9% of smokers agree with better public education concerning the dangers of smoking ($p< 0.001$). Finally, 59.8% of non-smokers vs 42.7% of smokers are in favour of instituting a publicity campaign against smoking ($p< 0.001$).

Conclusions: The overwhelming majority of non-smokers and most of the smokers agreed that some measures should be undertaken to curb tobacco use among the population. Smokers however were significantly less inclined to accept any form of restrictions on smoking. The most widely accepted measure was the prohibition of smoking in public areas.

Memory Stick

TOBACCO USE AND RISK AWARENESS AMONG A MOSTLY YOUNG ADULT POPULATION

T. Chimonas[1], P. Petrikos[2], E. Kostopoulos[2], **Eirini Fanouraki**[3], D. Panagiotakos[4], E. Chimonas[3], M. Elisaf[1]

[1]University of Ioannina, Ioannina, [2]251 Hellenic Airforce General Hospital, [3]Hellenic Atherosclerosis Society, [4]Harokopio University, Athens, Greece

Purpose: One of the most important risk factors for smoking is the general population's awareness of the dangers of tobacco. Therefore, we sought to explore the level of knowledge regarding tobacco-related health risks among a Greek population.

Methods: The study sample consisted of 2547 unselected adults, 90.9% male and 9.1% female, aged 18 to 65 (mean age 29.1±8.3 years old), belonging to the Hellenic Air Force personnel, who filled out an anonymous questionnaire.

Results: Among the participants, 49.0% were smokers. Almost all, 97.4% of smokers vs 98.5% of non-smokers, agreed that smoking is harmful (p=not significant); However, 12.9% of non-smokers vs 18.7% of smokers did not believe that it can cause lung disease (p< 0.001); 22% vs 30.4%, respectively, that it can cause cancer (p< 0.001); 40.6% vs 46.6% that it can cause heart disease (p=0.002); 46.4% vs 56.4% that it can cause premature death (p< 0.001) and 59.0% vs 63.0% that it can cause strokes. Participants who showed ignorance of the dangers of smoking are generally younger (e.g. 2.6 years younger regarding the question on heart disease, p< 0.001) and have significantly less years of academic training (0.4-0.8 years less, p< 0.05-0.001). Most agreed that smoking is addictive (87.6% of smokers vs 92.6% of non-smokers, p< 0.05).

Conclusions: The overwhelming majority agreed that smoking is generally hazardous for one's health, but many participants did not know what these hazards actually are. Smokers in particular tended to underestimate the dangers of smoking.

Memory Stick

BLOOD PRESSURE LEVELS CONSTITUTE THE MOST IMPORTANT DETERMINANT OF THE METABOLIC SYNDROME, IN A GREEK SAMPLE

Theodoros Chimonas[1], V. Athyros[2], A. Karagiannis[2], A. Achimastos[3], C. Pitsavos[3], M. Elisaf[1], D. Panagiotakos[4]

[1]University of Ioannina, Ioannina, [2]Aristotle University of Thessaloniki, Thessaloniki, [3]University of Athens, [4]Harokopio University, Athens, Greece

Purpose: The prevalence of various constituents of the metabolic syndrome (MS) may vary between different populations. Therefore, we sought to evaluate the determinants of the MS in a sample drawn from the Greek population.

Methods: A random sample of 824 male (55.7±11.1 years) and 1199 female (57.6±10.3 years) subjects with MS (NCEP ATPIII), but without diabetes mellitus or established cardiovascular disease, was selected from several Greek areas. Clinical and biochemical markers were measured. Besides descriptive analysis, principal components analysis (PCA) was applied to evaluate the inter-relationships between the inherent characteristics of the MS.

Results: Among the participants, 87.6% showed elevated BP levels, 79.9% hypertriglyceridemia, 62.6% low HDL-C levels, 71.4% impaired FG and 91.5% abdominal obesity. Moreover, 26.7% of the participants had all five criteria, followed by the combination of elevated BP levels, abdominal obesity, high FG and high triglycerides (14.2%). PCA revealed 3 main components that explained 68.4% of the total information. The first component was heavily loaded with BP measurements (28.6% of the total information explained), followed by a component loaded mostly with lipidemic variables (21.6%) and a component loaded with FG and waist measurements (18.1% of the total information). These three components represent the most important physiologic correlations between the five criteria of the MS.

Conclusion: The most dominant characteristic of our sample with MS was elevated BP levels, followed by dyslipidemia. Thus, targeting blood pressure levels in the general population may assist in better preventing metabolic syndrome and its sequelae.

Memory Stick

TYPE 2 DIABETES AND THE CORONARY ANGIOGRAPHIC STATE ARE MUTUALLY INDEPENDENT PREDICTORS OF FUTURE VASCULAR EVENTS AMONG ANGIOGRAPHIED CORONARY PATIENTS

Christoph H. Saely[1,2,3], T. Gansch[1], S. Greber[1], P. Rein[1,2,3], A. Vonbank[1,2,3], S. Beer[1,2,3], F. Schmid[1], T. Marte[1], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Background: Type 2 diabetes (T2DM) confers a strongly increased risk of vascular events. It is not certain to what extent the baseline CAD state accounts for the increased vascular risk of diabetic patients.

Methods: We enrolled 750 patients undergoing coronary angiography for the evaluation of stable CAD. At angiography, CAD was diagnosed in the presence of any irregularities of the vessel wall. Stenoses ≥50% were considered significant, the extent of CAD was defined as the number of significant stenoses. Vascular events were recorded over 8 years.

Results: The prevalence of CAD (87.8% vs. 80.4%; p=0.029) and of significant stenoses (69.5% vs. 58.4%; p=0.010) as well as the extent of CAD (1.7±1.5 vs. 1.4±1.5; p=0.014) were significantly higher in patients with T2DM (n=164) than in nondiabetic subjects (n=586). T2DM after multivariate adjustment strongly predicted vascular events (adjusted hazard ratio (HR) = 1.55 [1.17-2.05]; p=0.002). The presence of CAD (HR=3.59 [2.11-6.13]; p< 0.001), the presence of significant stenoses (HR=2.29 [1.70-3.09]; p< 0.001) and the extent of CAD (standardized adjusted HR=1.40 [1.25-1.56]; p< 0.001) significantly predicted vascular events. These characteristics still predicted vascular events after additional adjustment for T2DM (HR=3.46 [2.03-5.91], p< 0.001; 2.24 [1.66-3.02], p< 0.001; and 1.38 [1.24-1.54], p< 0.001, respectively). T2DM remained strongly and significantly predictive of future vascular events after adjustment for the presence and extent of CAD (HR=1.41 [1.07-1.87]; p=0.016).

Conclusions: Among coronary patients, the presence and the extent of CAD are higher in patients with T2DM than in nondiabetic individuals. T2DM and the baseline CAD state are mutually independent

Memory Stick

CORRELATION BETWEEN CAROTID ATHEROSCLEROTID DISEASE AND MULTI-VESSELS CORONARY DISEASE IN PATIENTS WITH ACUTE CORONARY SYNDROME

Alessia Azzano, F. Fini, A.L. Romano, G. Zampi, V. Paravati, C. Gaudio

Heart and Great Vessels, Sapienza University of Rome, Rome, Italy

Introduction: Many studies about Atherosclerosis have documented that carotid plaques is predictive of coronary involvement.

Objective: To value the possible correlation between the extension of atherosclerotic disease in the carotid and coronary district.

Materials and methods: Between January 2008 and May 2009, 191 patients with Acute Coronary Syndrome were underwent in-hospital coronary study and Eco-Color-Doppler of the epiaortic vessels. The pts positive to the Doppler were divided into two groups: Group A, 32 pts with only one carotid artery affected, and Group B, 116 pts with both carotid arteries affected.

Results: Among 191 pts underwent Eco-Color-Doppler of the epiaortic vessels, in 148 pts (77%) it was found a carotid artery involvement. In Group A 87.5% of pts have had a one-vessel coronary disease; 9.3% two-vessels disease and 3.1% three-vessels disease. In Group B 8.6% of pts have had an one-vessel disease; 34.4% two-vessels disease; 56.8% three-vessels disease.

Conclusions: The study have documented that greater is the number of carotid artery involved in the atherosclerotic disease and greater is the probability to find a coronary multi-vessels disease. Use of Carotid ultrasound improve the stratification of cardiovascular risk and predict presence and extent of coronary artery disease.

Memory Stick

INSULIN RESISTANCE IS ASSOCIATED WITH METABOLIC SYNDROME BUT NOT WITH ANGIOGRAPHICALLY DETERMINED CORONARY ARTERY DISEASE

Alexander Vonbank[1,2,3], C.H. Saely[1,2,3], P. Rein[1,2,3], S. Beer[1,2,3], M. Woess[1], J. Breuss[1], B. Gaensbacher[1], H. Drexel[1,2,3,4]

[1]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), [2]Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [3]Private University of the Pricipality of Liechtenstein, Triesen, Liechtenstein, [4]Drexel University College of Medicine, Philadelphia, PA, USA

Objectives: Insulin resistance (IR) is the key feature of the metabolic syndrome (MetS) and in prospective studies predicts atherothrombotic events. Its association with directly visualised coronary atherosclerosis is unclear. We hypothesised that IR is associated with both angiographically determined coronary artery disease (CAD) and with the MetS.

Methods: We enrolled 986 consecutive patients undergoing coronary angiography for the evaluation of suspected or established stable CAD; significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥50%. IR was determined by the HOMA index; the MetS was defined according to ATPIII criteria.

Results: HOMA IR scores were significantly higher in MetS patients than in subjects without the MetS (6.4±2.1 vs. 2.2±2.0; p< 0.001). In contrast HOMA-IR did not differ significantly between patients with significant CAD and those who did not have significant CAD (3.9±1.4 vs. 3.2±4; p=0.490). When both, the presence of MetS and of significant CAD were considered, HOMA-IR was significantly higher in patients with the MetS both among those who had significant CAD (7.2±2.8 vs. 2.3±2.1; p< 0.001) and among those who did not have significant CAD (5.3±5.7 vs. 2.1±1.4; p< 0.001) whereas it did not differ significantly between patients with significant CAD and subjects without significant CAD in patients with the MetS (7.2±2.8 vs. 5.3±5.7; p=0.679) nor in those without MetS (2.1±1.4 vs. 2.3±2.1; p= 0.411). Similar results were obtained with the IDF definition of the metabolic syndrome.

Conclusion: IR is significantly associated with the MetS but not with angiographically determined coronary atherosclerosis.

Memory Stick

ENDOTHELIAL PROGENITORS CELLS AND MICROPARTICLES IN HIV-INFECTED PATIENTS

Erika Ferrari Rafael Silva[1], F.A.H. Fonseca[2], C.N. França[2], P.R.A. Ferreira[1], C.M. Monteiro[2], L.M. Camargo[2], R. Salomão[1], S.B. Tenore[1], D.S. Lewi[1]

[1]Infectious Diseases, [2]Cardiology, Federal University of São Paulo, São Paulo, Brazil

Background: Nowadays the HIV infection is recognized as an inflammatory disease and can lead to endothelial disfunction. Endothelial injury plays a critical role in coronary artery disease, but the assessment of this injury has been problematical. Recently, it has been shown that endothelial cells (EC) release microparticles (MP) on activation or apoptosis and that and evaluation of MP can provide useful information on EC status in patients with an increase cardiovascular risk.

Methods: We studied endothelial progenitors (EPC) cells and MP in HIV-infected naive patients and compared with HIV negative controls. Standard laboratory study included: lipid profile, glycaemia, C-reactive protein and apolipoprotein B. The EPC cells and MP were measure by flow citometry. The EPC were identified using the following markers: CD34+, KDR for definition and CD133+ for immature lineage cells. The MP were characterized with CD31+, CD42+ and CD51+.

Results: Thirty patients were included, 15 in each group, 73,3% were male with mean age 30,9 years. The lipid profile was significant only in HDL-c and LDL-c between the groups. In the HIV-infected group we observed 0,01% of CD34+/KDR+ and it was not isolated CD34+/CD133+ neither CD133+/KDR+. In this group it was also observed more release of MP CD51+ and CD31+/CD42+ comparing to the control group, any MP CD31+/CD42- were found.

Conclusion: Our results suggest a possible imbalance between EPC and MP. This new finding in HIV-naive patient may be associated with increased cardiovascular risk in the long term follow-up, and can be aggravated after antiretroviral therapy.

Memory Stick

B2-MICROGLOBULIN, A NOVEL BIOMARKER OF PERIPHERAL ARTERIAL DISEASE, IS INDEPENDENTLY ASSOCIATED WITH AORTIC STIFFNESS IN THESE PATIENTS

Jaak Kals, M. Zagura, M. Serg, P. Kampus, K. Zilmer, E. Unt, J. Lieberg, J. Eha, A. Peetsalu, M. Zilmer

University of Tartu, Tartu, Estonia

Objective: Arterial stiffness is a prominent feature of vascular ageing and strongly predicts cardiovascular and total mortality. The β2-microglobulin, (β2M) a newly identified biomarker of peripheral arterial disease (PAD), is related to renal insufficiency, inflammatory and neoplastic diseases, but may also play a role in vascular dysfunction. However, the relationship between arterial stiffness and β2M has not been previously studied in patients with atherosclerosis. In the present study we examined possible association between β2M and arterial stiffness in patients with PAD and in healthy subjects.

Methods: Plasma β2M levels and parameters of arterial stiffness such as aortic pulse wave velocity (aPWV) and augmentation index (AIx) and were measured in 66 patients with PAD and in 66 apparently healthy subjects.

Results: Plasma levels of β2M, aPWV and AIx were significantly increased in patients with PAD compared with controls (1858.1±472.8 vs 1554.5±277.9 µg/L, P< 0.001; 9.9±2.2 m/s vs 7.6±1.6 m/s, P< 0.001; 28±8 vs 14±11 %, P< 0.001; respectively). There existed significant correlation between aPWV and β2M for the patient group (R=0.47; P< 0.001), but not for the controls (R=0.14; P=0.26). In multivariate analysis, β2M remained independently associated with aPWV, fetuin-A, age and glomerular filtration rate in patients (R^2=0.5, P< 0.001). We found no relationship between β2M and AIx in either group.

Conclusion: We demonstrated that among patients with PAD elevated plasma β2M levels were associated with higher aortic stiffness irrespective of distending pressure and cardiovascular disease risk factors. These data suggest that β2M may be involved in the pathogenesis of aortic stiffness in atherosclerosis.

Memory Stick

DYSLIPIDEMIA AND CARDIOVASCULAR RISK IN NORTHERN GREECE. ATHOS CARDIO GREECE STUDY

Apostolos Efthimiadis, I. Kanonidis, G. Sakantamis, I. Efthimiadis, E. Paschalidou, S. Pavlidou, A. Tavridou

Northern Greece Atherosclerosis Society, Thessaloniki, Greece

Objective: To study the prevalence of dyslipidemia and its relation to cardiovascular risk in a Northern Greek population sample.

Methods: 3000 subjects (54% males and 46% females) with mean age 62±13 years from Northern Greece were examined during an epidemiological project the last three years. All subjects gave informed consent form and completed a questionnaire on personal and family medical history. Demographic/anthropometric characteristics and blood pressure were recorded. Biochemical parameters (total cholesterol, HDL, LDL, triglycerides) were measured by the Cholestech Kit (dry chemistry method). The study sample was divided into two groups.

Group A: Patients receiving lipid lowering agents and/or a having LDL>160 mg/dl.

Group B: Patients with LDL≤160 mg/dl not receiving lipid lowering treatment. SPSS 15.0 was used for the epidemiological analysis.

Data are presented as mean±standard deviation.

Results: Dyslipidemia was diagnosed in 54.6% of the subjects. 42% of the dyslipidemic group was not receiving lipid lowering agents. BMI and waist circumference were slightly higher over the dyslipidemics (28.2±4 kg/m² and 97.4±13 cm respectively) whereas hypertension, smoking and diabetes found to cluster in the same group.Cardiovascular risk score was 19% for the dyslipidemics and 29% for the dyslipidemics who smoked. Atheromatic index was 4.7±1.6 for the dyslipidemic group.

Conclusions: Dyslipidemia is a major cardiovascular risk factor. Despite that fact most subjects with dyslipidemia were not receiving lipid lowering therapy. The prevalence of risk factors was increased in the subjects with dyslipidemia and those were at higher risk of developing cardiovascular disease.

Memory Stick

SMOKING AND CARDIOVASCULAR RISK IN NORTHERN GREECE. ATHOS CARDIO GREECE STUDY

Apostolos Efthimiadis, A. Tavridou, I. Kanonidis, I. Efthimiadis, E. Paschalidou, S. Pavlidou, G. Sakantamis

Northern Greece Atherosclerosis Society, Thessaloniki, Greece

Objective: To study the relation between smoking habit and cardiovascular risk in a Northern Greek population sample.

Method: 3000 subjects (54% males and 46% females) with mean age 62±13 years from Northern Greece were examined during an epidemiological project the last three years. All subjects gave informed consent form and completed a questionnaire on personal and family medical history. Demographic/anthropometric characteristics and blood pressure were recorded. Biochemical parameters (total cholesterol, HDL, LDL, triglycerides) were measured by the Cholestech Kit (dry chemistry method). All subjects were divided into three groups according to smoking habit: current smokers, ex smokers and non smokers. SPSS 15.0 was used for the epidemiological analysis. Data are presented as mean±standard deviation.

Results: Most of the subjects studied were non smokers (74.1%). Smoking habit was more frequent in men than in women (65/35% respectively) and in younger ages (47±14 years) with a mean smoking duration of 16±6 years. Dyslipidemia and hypertension were the strongest risk factors in all three groups. Atheromatic index and cardiovascular risk score were higher in the current smokers group (4.7±1.6 and 21% respectively).

Conclusions: Smoking habit found to be strongly related to the overall cardiovascular risk whereas smoking cessation reduced it greatly. Most of the ex smokers had quit smoking due to health problems such as diabetes, dyslipidemia, coronary artery disease and hypertension. Today every smoker should be considered as a patient examined thoroughly for the existence of other risk factors and be advised to follow a smoking cessation program.

Memory Stick

EFFECTS OF FOOD ON POSTPRANDIAL BLOOD PRESSURE AND ARTERIAL STIFFNESS MEASUREMENT

Madeleine Ball[1], I. Robertson[1,2], K. Ahuja[1]

[1]School of Human Life Sciences, University of Tasmania, [2]Clifford Craig Medical Research Trust, Launceston, TAS, Australia

Background: Recent research suggests central pulse pressure may be a better indicator of cardiovascular disease outcomes than brachial pressure. Little information is available about the effect of food on postprandial central pressure and arterial stiffness measures made using non-invasive pulse wave analysis (PWA).

Objective: To investigate the effects of water and food plus water intake on brachial and central blood pressure (BP) and measures of arterial stiffness, including augmentation pressure (AP) and index (AI) using PWA.

Design: 35 subjects had BP and PWA measured fasting and for two hours after the intake of either water or breakfast (1300 kJ) in random order.

Results: Baseline fasting measures of BP and arterial stiffness were not significantly different before the two interventions. Consumption of food plus water, compared to water alone, led to a significantly lower (all $p < 0.01$) brachial diastolic pressure (Δ -3.8mmHg), central BP (Δ systolic -6.1mmHg; Δ diastolic -3.8mmHg), central pulse pressure (Δ -2.4mmHg), mean arterial pressure (Δ -4.6mmHg), AP (Δ -2.9mmHg) and AI (Δ -5.3%).

Conclusion: Markers of central hemodynamics are sensitive to feeding state and should therefore be measured fasting to avoid variability due to recent (1-2 hours) food intake. This is particularly important where measurements are repeated over time to assess the effect of medication and lifestyle changes on CHD risk factors.

Memory Stick

PREVALENCE OF THE CAROTID-INTIMA-MEDIA THICKNESS AND CAROTID PLAQUES IN HYPERTENSIVE PATIENTS

Jorge Ble-Castillo[1], L. Cantera-Rangel[2], A. Garcia-Magaña[3], F. Aguilar-Balcazar[4], H. Ble-Castillo[5], S. Aguilar-Barojas[1], I. Juarez-Rojop[1], A. Rodriguez-Hernández[1], H. Aguilar-Mariscal[1], J. Díaz-Zagoya[1]

[1]Centro de Investigacion, Universidad Juarez Autonoma de Tabasco, [2]Cardiologia, Hospital General de Zona 46, Instituto Mexicano del Seguro Social, [3]Cardiologia, [4]Imagenologia, Hospital General de Zona 46, IMSS, [5]Urgencias, Hospital del Sureste, Villahermosa, Mexico

Objective: Investigate the prevalence of subclinical atherosclerosis measured by carotid intima-media thickness (CIMT) and carotid plaques (CPs) in patients with hypertension (HTA).

Methods: A prospective, transversal study was carried out from June to August 2008. 141 patients, 40-65 years aged, with HTA and low and intermediate cardiovascular risk (CVR) according to Framingham cardiovascular risk score (FCRS) were included. Mode B carotid ultrasonography (USG) was performed.

Results: A hundred women and 41 men were included. 82.3% subjects were calculated on low risk while 17.7% were on intermediate risk. Subclinical atherosclerosis by USG was present in 30% of patients, 92.8% of them had CP and 7.2 % exhibited CIMT greater than 0.9 mm. Intermediate CVR subjects had three times more probability to have CP than low CVR patients. Twice more probability to have CP in non-controlled than in controlled patients was observed. A positive correlation between CIMT and CVR was found with r= 0.343 (p< 0.0001) for the right carotid and r= 0.1902 (p= 0.024) for the left carotid. The correlation between the CIMT and the systolic blood pressure (SBP) was positive: r= 0.172 (p= 0.0412) and r= 0.351 (p< 0.0001) for right and left carotid respectively.

Conclusions: A high prevalence of subclinical atherosclerosis was found by the CIMT and CPs measured by USG in hypertensive patients. The use of CIMT and CPs could be recommended to improve Framingham stratification criteria in these subjects.

Memory Stick

SERUM ATHEROGENIC LIPID MARKERS IN SUBJECTS FROM THREE ISLANDS OF THE AZORES´ ARCHIPELAGO (PORTUGAL) - A COMPARISON STUDY

Rita Ferin[1,2], C. Correia[1], M. Raposo[2], A.R. Castro[1], C. Reis[1], T. Vieira[1], B. Gonçalves[1], M.L. Pavão[1,2]

[1]DCTD, [2]CIRN, University of the Azores, Ponta Delgada, Portugal

São Miguel (SM), Graciosa (GR) and São Jorge (SJ) are three islands of the Azores' Archipelago, where the death rate from coronary artery disease is about twice than in mainland Portugal. The aim of this study was to evaluate and compare serum atherogenic lipid markers as reflected by lipid profile, as well as by apoB/apoA-1 ratio in apparently healthy subjects from those islands. The study group was formed by 321 subjects with no chronic diseases, aged 20 to 60 years, born living in the respective island (156 from SM, 92 from GR and 73 from SJ). In all cases, about 64% of subjects were hyperlipidemic, mainly hypercholesterolemic. LDL-C and HDL-C concentrations were respectively, lower and higher in SM than in SJ or GR. ApoB concentration was 40% and 17% higher in SJ than in SM and GR, respectively, while apoA-1 reached the maximum in SM (178±38mg/dL).Regarding apoB/apoA-1 ratio, taken as a better atherogenic marker then conventional lipid profile, subjects from GR exhibited the highest mean value (0.85±0.4), followed by those from SJ (0.75±0.3) and SM (0.58±0.2). Particularly in men from GR and SJ, ratios were 1.0 and 0.9, respectively, which corresponds to a high risk of developing a cardiovascular incident.

Memory Stick

RISK FACTORS PREVALENCE AND CONTROL IN SECONDARY PREVENTION AFTER MYOCARDIAL INFARCTION AND ISCHEMIC STROKE IN UKRAINIAN URBAN AREA

A. Yagensky[1], **Iryna Sichkaruk**[1], N. Sydor[2], J. Kyyak[2], E. Zaremba[2], L. Dukhnevych[1]

[1]Lutsk City Hospital, Lutsk, [2]Lviv National Medical University, Lviv, Ukraine

Objectives: In Ukraine cardiovascular morbidity and mortality is one of the highest in Europe. Lack of risk factors (RF) control in primary and secondary prevention may play major role in this situation.

Methods: RF prevalence and control was assessed in representative sample of 235 patients (pts) after ischemic stroke (PostIS) and 312 pts after myocardial infarction (PostMI) randomly selected from 2229 pts discharged from hospital between 2000 and 2005 in Ukrainian city Lutsk.

Results: As shown in table the most prevalent RF in both groups was arterial hypertension (AH). Only 11,9% of PostIS and 21,3% PostMI pts had their BP below 140/90 mm Hg (p< 0,001). Higher AH prevalence in PostIS pts corresponded with higher levels of BP. No difference between groups was found in other RF as well as lipids, glucose and high-sensitivity CRP levels. Insufficient number of pts took antithrombotic drugs, especially in PostIS group: 39,1% vs 52,6% in PostIM (p=0,008) and very few took statins: 1,7% PostIS vs 11,2% PostMI (p< 0,001).

Conclusions: High prevalence of RF with poor control was found in pts after ischemic events in Ukrainian city Lutsk. PostIS patients had higher prevalence of AH and worse BP control. Inappropriately low number of patients receive preventive treatment especially in PostIS group

	AH	Diabetes	Obesity	Abdominal obesity	Hypercholesterolemia	Smoking
PostMI	84,3	13,5	44,8	57,6	76,1	18,0
PostIS	89,8	15,3	44,2	60,6	73,2	18,3

[Prevalence of RF in PostMI and PostIS pts (%)]

Memory Stick

ADIPOSE TISSUE INFLAMMATORY ACTIVITY IS ASSOCIATED WITH CARDIOVASCULAR DISEASE

T. Weiss[1], I. Seljeflot[2], E. Hjerkinn[2], **Harald Arnesen**[2]

[1]Medical Division, Dept for Cardiology, [2]Centre for Clinical Heart Research, Oslo University Hospital, Ulleval, Oslo, Norway

Background: Obese patients are at higher risk of developing cardiovascular disease (CVD). Several studies suggest obesity as an independent risk factor. Adipose tissue is now accepted as an endocrine organ that produces and secrets a variety of cytokines, hormones and other metabolites. Among this versatile group of mediators and effectors of inflammation, we have chosen to study the expression of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), plasminogen activator inhibitor-1 (PAI-1), interleukin-18 (IL-18) and interleukin-6 (IL-6). All these adipokines have in their circulatory form been associated with cardiovascular disease. However, there is not much data available on their expression in adipose tissue in human subjects with and without cardiovascular disease.

Methods: We successfully isolated RNA from subcutaneous fat biopsies of 61 patients with or without cardiovascular disease. We then measured the RNA expression of MMP-9, TIMP-1, PAI-1, IL-18 and IL-6 with Real-Time PCR, using relative quantification.

Results: All inflammatory mediators, except IL-18 were higher expressed in patients with cardiovascular disease compared to those without. Pooling the gene expression data, trying to capture the overall inflammatory activity in the adipose tissue, we observed a highly significant association with CVD.

Conclusion: Trying to capture the activity in addition to the mass of adipose tissue could provide useful hints towards presence of cardiovascular disease.

Memory Stick

INTERRELATIONSHIP BETWEEN PULSE WAVE VELOCITY AND RISK FACTORS OF ATHEROSCLEROSIS IN YOUNG HEALTHY PERSONS

Tatyana Pronko

Grodno State Medical University, Grodno, Belarus

Pulse-wave velocity is a useful non-invasive index to assess arteriosclerosis. The most frequent risk factors of atherosclerosis are smoking and overweight.The aim of the study was to define the correlations between pulse wave velocity (PWV) and risk factors of atherosclerosis in young healthy persons.

Methods: The study population included 62 healthy volunteers (37 men 25 women), average age 26.0±2.4 years. Patients were divided into 4 groups: group A includes 16 healthy persons without risk factors of atherosclerosis, group B includes 18 active smokers, group C - 13 overweight persons, group D - 15 active smokers with overweight. Carotid femoral PWV was measured noninvasively.

Results: The body mass index (BMI) was 23.19±1.3, PWV was 5.41±0.73 m/s in group A. Smokers have BMI 22.3±1.7. PWV was on 58% higher (8.58±0.88 m/s, ($p< 0.05$)) to compare with group A. The BMI in group C was 29.18±3.2, PWV was 8.05±0.94 m/s, what is on 48% higher ($p< 0.05$) to compare with group A. The BMI in group D was 28.93±3.8, PWV was 8.66±0.67 m/s, what is on 60% higher ($p< 0.05$) to compare with group A. PWV positively correlated with smoking ($r=0.36$; $p< 0.05$) and with BMI ($r=0.41$; $p< 0.05$).

Conclusions: Smoking and overweight increase PWV in healthy young persons. Non-invasive measurement of carotid femoral PWV can be used in clinical practice in assessment of pre-clinical atherosclerosis and detect groups of high cardiovascular risk.

Memory Stick

FASTING BLOOD GLUCOSE LEVELS CORRELATE WITH SEVERITY OF CAROTID STENOSIS IN NON-DIABETIC HYPERLIPIDEMIC PATIENTS

Elias Skopelitis, D. Levisianou, S. Kougialis, N. Iliopoulos, T. Gogalis, A. Kroustalis, G. Groumas, E. Katsadorou, P. Terlis, A. Destounis, C. Drakoulis, C. Mpilinis

Agios Panteleimon General Hospital, Nikea, Greece

Background and aims: The correlation between fasting blood glucose (FBG) levels and cardiovascular mortality in non-diabetic individuals is well established. Also, carotid stenosis (CS) is a risk marker for cardiovascular disease in the general population. The relation between FBG and CS in nod-diabetics is not fully elucidated.

Materials and methods: A pilot cross-sectional survey of 43 consecutive patients coming for follow-up visit in a single lipid outpatient clinic (74.4% males, mean age 69.0±8.41 years) with carotid stenosis and no history of diabetes was launched. Anthropometrics, risk factors, complete lipid profile, and CRP were obtained at enrollment. Oral glucose tolerance test (OGTT) was performed in every patient. Carotid stenosis was evaluated by ultrasonography.

Results: The 55.8% had a history of active smoking, 60.5% hypertension and 53.5% cardiovascular disease. Total cholesterol was 223.51±43.92 mg/dl, triglycerides 123.2±54.24 mg/dl, HDL 56.25±11.9 mg/dl, LDL 142.93±37.24 mg/dl, CRP 2.89±3.77, BMI 26.67±2.71kg/m^2 and carotid stenosis 27.9±24.1%. Fasting blood glucose was significantly correlated with carotid stenosis ($r=0.359$, $p=0.018$). Of the examined risk factors in multivariable analysis, only fasting blood glucose ($\beta=0.698$, $p=0.018$) and smoking ($\beta=6.48$, $p=0.046$) were independently correlated with carotid stenosis.

Conclusion: Fasting blood glucose levels were associated with degree of carotid stenosis in non-diabetic, hyperlipidemic patients. In such patients routine evaluation of carotid stenosis for the stratification of the cardiovascular risk may be considered. Also, a strict management of other risk factors is necessary in order to prevent cardiovascular morbidity.

Memory Stick

THE STUDY OF HEREDITARY FACTORS, PREDISPOSING TO THE DEVELOPMENT OF ANTIATHEROGENIC PHENOTYPE IN IV PATIENTS, ON EXAMPLE OF APOE-GENE POLYMORPHISM

Alexandra Allahverdyan[1], J. Maksimova[2], E. Schakhtschneider[1], S. Malyutina[1], V. Maksimov[1]

[1]Institute of Internal Medicine SB RAMS, [2]Novosibirsk State Medical University, Novosibirsk, Russia

Cardiovascular disease is the leading killer for humanity.

Ichthyosis Vulgaris (IV) - is an autosomal dominant disorder, in which observed skin and lipid metabolism changes. IV patients have lower level of total cholesterol in plasma, lower IBM, than in the population. Obesity, arterial hypertension, ischemic heart disease, diabetes mellitus are extremely rarer too. This gave us a reason to think that the patients with IV can have antiatherogenic phenotype.

Objective: The goal of this work was to study the allele frequencies of Apolipoprotein E Gene Polymorphisms in the Protein Coding Region in IV patients in comparison to the control group.

Material and methods: Two independent groups were tested. First group consist of the patients with IV (74 men). As a control group, the samples of 25-64 year-old residents of Novosibirsk (881 men) were examined in the framework of the WHO program MONICA. DNA extraction and genotyping were performed by standard protocols.

Results: We found increased frequency of e2 allele (15% in patients against 7% in a population), due to genotypes e2/e2 (4 person - 5.4%) and e2/e3 (10 person - 13,5%). The distribution of genotype frequencies are not in Hardy-Weinberg equilibrium. Among 881 samples from the control group we found no genotype e2/e2.

Conclusions: The genotype distribution did not meet Hardy-Weinberg equilibrium, by increasing the e2 allele. Probably, this is due to the conservation of rare genotypes among IV patients. To clarify the cause of this phenomenon, we continue research.

This work was supported by President RF grant [1]MK-2666.2009.7.

Memory Stick

FOOD, QUALITY OF A LIFE AND ABDOMINAL OBESITY

Oksana Bragina, L. Shcherbakova, G. Simonova

Institute of Internal Medicine SB RAMN, Novosibirsk, Russia

Introduction: There is prevalence of persons with overweight and obesity in Russia for last decades.Especially dangerous abdominal obesity that promotes of development of cardiovascular diseases, a diabetes, cancer.

The purpose: To study consumption of food and indicators of quality of a life at inhabitants of Novosibirsk, 45-69 years with and without abdominal obesity (Project HAPIEE- Determinants of cardiovascular diseases in the Eastern Europe).

Materials: The food was estimated using food frequency questionnaire (142 names), the quality of a life was estimated by questionnaire SF-36. Waist circumferences were ≥ 102 cm for men and ≥ 88 cm for women (NCEP ATP III, 2004). Statistical data were analyzed by using SPSS 11.0

Result: It has been established that women with abdominal obesity consumed less carbohydrates, starch, sugar, food fiber than women without abdominal obesity. In group of women with abdominal obesity were three folders more women with diabetes who be special dieting. Men with abdominal obesity consumed more daily food energy, total fat than men without abdominal obesity. Women with abdominal obesity had lower indicators of physical functioning and viability and role emotional functioning. Men with abdominal obesity had low indicators of a physical component of health and social functioning.The

Conclusion: abdominal obesity connection with a food and indicators of quality of life. The hyper calorie food often promotes development abdominal obesity. The abdominal obesity promotes decrease physical functioning and then reduces some indicators of mental functioning.

Memory Stick

THE PREVALENCE OF MASKED HYPERTENSION IN TREATED TYPE 2 DIABETIC PATIENTS AND ITS ASSOCIATION WITH CARDIOVASCULAR TARGET ORGAN DAMAGE

Jiri Charvat, J. Chlumský, M. Szabó, V. Zamrazil

Medical, Faculty Hospital, Charles University, Prague, Czech Republic

Objectives: To evaluate the prevalence of masked hypertension (MH) in treated type 2 diabetic patients and its association with cardiovascular target organ damage.

Methods: Using 24-hours BP monitoring, tissue Doppler echocardiography and carotid sonography we evaluated 64 type 2 diabetic patients without history of cardiovacular events however treated for hypertension. The patients with controlled BP according to office measurement (≤130/85 mm Hg) were examined. MH was diagnosed if daily 24-hour BP≥ 135/85 mm Hg. The association of MH with micro/macroalbuminuria, left ventricle function carotid artery parameters and postischemic dilatation of brachial artery was evaluated.

Results: MH was diagnosed in 24 patients (37.5%). Body mass index (BMI) was significantly higher in patients with MH (33.3±2.7 kg/m^2) than without MH (30.1± 2.6 kg/m^2, p=0.005). Number of patients with micro/macroalbuminuria was higher in patients with MH - 9 (37.5%) vs. 4 patients without MH (10%, p=0.008).

Multivariate regression analysis confirmed the correlation of MH with BMI and micro/macroalbuminuria but revealed also the association of MH with parameter of diastolic function E/E´ (r=0,431, p< 0.05) and with intimomedial thickness (IMT) of carotid artery (r=0,557, p< 0.05)

Conclusion: Masked hypertension in treated type 2 diabetic patients is frequent and significantly associated with BMI, micro/macroalbuminuria, IMT of carotid artery and diastolic function parameter E/E´.

Supported by grant IGA MZ ČR NR/9520-3

Memory Stick

FTO POLYMORPHISM IS ASSOCIATED WITH MYOCARDIAL INFARCTION

Jaroslav Hubacek[1,2], for the GENDEMIP Research Group

[1]*Institute for Clinical and Experimental Medicine,* [2]*Centre for Cardiovascular Research, Prague, Czech Republic*

Introduction: Myocardial infarction (MI) is the most common cause of death in industrial countries. All general MI risk factors have some genetic components and the proportion of genetic predisposition is between 30-60 %. FTO gene („fat mass and obesity related gene", DNA demethylase) and its variants have been described primarily like important determinant of body weight. Further, FTO gene variant increases general mortality independent of fatness in men. We have analysed the putative association between FTO SNP rs17817449 and risk of MI development on middle European Caucasians.

Methods: *FTO* SNP rs17817449 (G → C) was analysed using PCR-RFLP in 1 191 representatively selected controls (age below 65) and 1 092 consecutive MI patients (age below 65). ANOVA and chi-square were used for statistical analysis.

Results: *FTO* rs17817449 has significant effect on body mass index (kg/m^2) both in controls (GG - 28.7 ± 3.7; GT - 28.3 ± 4.1; TT - 27.8 ± 3.9; P = 0.014) and in patients (GG - 29.2 ± 4.1; GT - 28.3 ± 4.5; TT - 28.2 ± 3.8; P = 0.006). Total cholesterol, HDL cholesterol, triglycerides, blood pressure were not associated with *FTO* variant. Further, the frequency of GG homozygotes is significantly higher in MI patients than between controls (21.4% vs 15.9%, P = 0.005; OR 1.38; 95% CI 1.10 - 1.73).

Conclusion: FTO rs17817449 (G → C in first intron) SNP is newly recognized genetic risk factor for myocardial infarction development.

Supported by project No. 1M0510 (MEYS, CR)

Memory Stick

LIPID PROFILE OF SPANISH PATIENTS WITH CORONARY HEART DISEASE TREATED WITH STATINS. THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], C. Guijarro[3], E. Alegria[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hopital Clinco Universitario, Santiago de Compostela, Santiago de Compostela, [3]Hospital de Alcorcon, Alcorcon, [4]Clinica Universitaria de Pamplona, Pamplona, [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Introduction: LDL-C is the therapeutic target for cardiovascular (CV) prevention and main CV guidelines recommend statins to reduce LDL-C levels. Patients with coronary heart disease (CHD) are high CV risk patients and have a lower LDL-C target. Some studies have found that low HDL-C and high triglycerides(TG) may have a role in coronary risk but statins have limited action on them. In this analysis we describe the lipid profile of Spanish statin-treated patients with CHD.

Methods: Analysis of 3710 Spanish statin-treated patients included in DYSIS(22063 patients in Europe and Canada).We used the ATP-III recommendations to classify patient´s risk and define the LDL-C goal and normality or not of the HDL-C and TG levels.

Results: In 3710 patients,23.8% were diagnosed with CHD(68.0% men, 27.6% women, p< 0,001).In this subpopulation: 75.1% hypertensive, 44.8% diabetics, 16.8% smokers and 25.6% had a family history of premature CHD. LDL-C was not at goal in 46.9% of patients with CHD vs. 51.4% of patients without CHD(p< 0.05).The prevalence of low HDL-C was 34.9%, higher compared to patients without CHD (29.8%, p< 0.001) and TG were elevated in 35.1% comparing to 38.4% in patients without CHD.

Conclusions: In this analysis of CHD patients, despite better control of LDL-C and TG than the total Spanish population studied, almost 50% of CHD patients do not reach LDL-C target.The prevalence of low HDL-C in these patients is significantly higher and 1/3 show high TG.A considerable number of CHD patients had an abnormal lipid profile.Is necessary use new therapies focus to treat complete lipid profile(LDL-C, HDL-C and TG).

Memory Stick

LEPTIN, ADIPONECTIN AND LEPTIN TO ADIPONECTIN RATIO IN DEPRESSIVE WOMEN

Miroslav Zeman[1], R. Jirák[2], M. Vecka[1], A. Žák[1], J. Macášek[1], E. Tvrzická[1], T. Vařeka[1], L. Vávrová[1], J. Kodydková[1], B. Staňková[1]

[1]IVth Dept Int Med, [2]1Psychiatric Dept, 1st Medical Faculty, Charles University, Prague, Czech Republic

Background: Depressive disorder (DD) is associated with an increased risk of type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD). It was suggested that metabolic syndrome (MetS), cluster of metabolic and hormonal changes, such as insulin resistance (IR), abdominal obesity, dyslipidemia, arterial hypertension and elevated glycaemia, could stand behind the connection. Recent findings have shown that leptin and adiponectin might play a role in both depression and MetS.

Materials and methods: The plasma leptin, adiponectin, parameters of lipid and glucose homeostasis and indices of IR were investigated in group of 38 women with DD. The results were compared with those of 38 healthy women of control group, matched for age.

Results: Women of DD group differed from those of control group in higher concentrations of plasma leptin (21.80±13.28 vs. 14.12±6.25) and the leptin-to-adiponectin ratio (1.80±1.12 vs. 1.04±0.72), both $P< 0.05$. Concomitantly, they had higher plasma TG (1.60±1.06 vs. 1.19±0.28 mmol/l ($P< 0.05$), insulin (13.38±8.0 vs. 7.8±3.8, C-peptide (0.84±0.31 vs. 0.66±0.19), both $P< 0.01$), microalbuminuria (12.65±10.18 vs. 4.49±3.07, $P< 0.05$) and higher value of the HOMA-IR index ($P< 0.01$).

Conclusions: In the presented pilot study, we found increased levels of plasma leptin in the group of depressive women and also the increased value of leptin-to-adiponectin ratio plasma levels and certain features of MetS. This could be the factor connecting depression with an increased risk of either DM2 or CVD. Moreover, HAM-D score of DD cases correlated negatively with adiponectin concentrations ($P< 0.01$).

Study was supported by the Research Project MSM 0021620820.

Memory Stick

WAIST CIRCUMFERENCE NEGATIVE CORRELATION WITH TESTOSTERONE AND SHBG IN YOUTH WITH PRE-METABOLIC AND METABOLIC SYNDROMES

Vesna Dimitrijevic-Sreckovic[1], P. Djordjevic[1], S. Damjanovic[1], B. Sreckovic[2], V. Barac[3], I. Soldatovic[4], M. Cvetkovic[1]

[1]Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [2]Bezanijska Kosa Clinical Center, [3]Institute of Medical Biochemistry, [4]Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia

Background: Visceral obesity, in fact a metabolic syndrome (MS), is related to decreased testosterone and sex hormone-binding globulin (SHBG) in adults. Peripheral conversion of testosterone to estrogen in excess peripheral adipose tissue may lead to secondary hypogonadism through hypothalamic-pituitary-gonadal axis inhibition. Aims of the study were to examine sex hormones in obese young males and correlations with visceral obesity, lipid status and blood pressure.

Methods: The study included 44 obese male individuals aged 16-30. Three of the following five criteria were used for MS diagnosis: waist circumference >90Pct; triglycerides >1.7mmol/l; HDL-cholesterol< 1.0mmol/l; hypertension>90Pct; glycemia>6.0mmol/L. Patients with less than three afore mentioned criteria were considered pre-MS. Testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and SHBG was determined by radioimmunoassay (RIA).

Results: Decreased testosterone (< 12.0nmol/l) was found in 11.3% obese young males (8.1±3.1nmol/l), with low SHBG (10.6±4.5) and normal FSH, LH and estradiol. Patients with pre-MS had normal testosterone (17.3±4.3nmol/l), FSH (3.84±2.25mU/l), LH (3.4±1.79mU/l), estradiol (110.0 ±51.8pmol/l) and SHBG (42.5±9.4) Patients with MS had normal testosterone (17.0±6.4nmol/l), FSH (3.38±2.17mU/l), LH (3.0±1.6mU/l), estradiol (115.3 ±48.4pmol/l), and SHBG at lower limits (17.8±8.4). Correlations: WC negative with testosterone and SHBG ($p < 0.05$); SHBG negative with BMI ($p < 0.05$), systolic and diastolic blood pressure ($p < 0.01$); estradiol positive with total cholesterol ($p < 0.01$), LDL-cholesterol and diastolic blood pressure ($p < 0.05$).

Conclusion: Negative correlation of waist circumference with testosterone and SHBG and of SHBG with BMI and blood pressure, proves the important effect of visceral obesity and insulin resistance on possible occurrence of secondary hypogonadism and infertility.

Memory Stick

ASSOCIATION OF THE Q223R POLYMORPHISM IN THE LEPTIN RECEPTOR GENE WITH AGE AT MENARCHE

Alicia García Anguita, P. Riestra, C. Garcés

Lipid Metabolism Laboratory, Fundación Jimenez Diaz, Madrid, Spain

Introduction: Energy regulating system plays an essential role in the pubertal development of females. Leptin, in addition to its role in body weight regulation, appears to be of critical importance on the onset of sexual maturation in children, in fact it is seems to be related to the age at menarche (AAM).

Objective: The aim of this study is to analyze the relationship of the polymorphism Q223R in the leptin receptor gene (LEPR Q223R) , which has been associated with leptin levels and body composition, to the AAM in a population-based sample of healthy pubertal girls in Spain.

Materials and methods: The study included 338 girls aged 11-17 years. Data on age at menarche was collected from girls. The Q223R LEPR polymorphisms were determined by TaqMan® allelic discrimination assays.

Results: Girls carrying the RR genotype have a significantly lower AAM (11.5 years) than those carries of the QR (11.9 years) or QQ genotypes (12.0 years) ($p< 0.05$). Furthermore, we found a significantly higher frequency of the RR genotype in girls with an AAM ≤ 11 than in girls with an AAM >13 (23.9% vs 7.8%, X^2: 11.17/ p: 0.0008). Also, the RR genotype frequency in girls with AAM of 12 was significantly higher than in those girls with an AAM >13 (16.8% vs 7.8%, X^2: 3.97/ p: 0.046).

Conclusions: The Q223R polymorphism in the LEPR gene is associated with a different age at menarche in Spanish girls, with the RR genotype being related to the earlier onset of menarche.

Memory Stick

NEGATIVE PSYCHOSOCIAL RISK FACTORS AND OBESITY. THE RESULTS FROM NATIONAL HEALTH SURVEY (WOBASZ)

Jerzy Piwonski, E. Sygnowska, A. Piwonska

Department of Cardiovascular Diseases Epidemiology, Prevention and Promotion of Health, Institute of Cardiology, Warsaw, Poland

Objective: Negative psychosocial risk factors can be both risk factors of obesity as well as can be a result of being obese.

Aim: to evaluate depressive symptoms (DS) and low social support level (SSL) in obese persons and the relation between DS, SSL and obesity.

Methods: the representative sample of polish population, 6392 men and 7153 women, aged 20-74, were examined in 2003-2005 in the frame of National Multicenter Health Survey (WOBASZ). DS were assessed using Beck's depressive scale and SSL using Berkman and Syme questionnaire.

Results: 1313 men -M (21%) and 1612 women - W (22%) were obese. Both M and W with obesity compared to not obese persons more often had DS (M - 29% vs 22%, $p< 0.0001$; W - 46% vs 30%, $p< 0.0001$). Low SSL were observed in 58% of obese M and 79% obese W (compared to 64% M and 68% W without obesity). The chance of getting depressive symptoms (independently of age) by obese person compared to not obese one was higher by 14% ($OR_{OD}=1.14$, $p=0.05$) in M, and in W even by 40% ($OR_{OD}=1.40$, $p< 0.0001$). The chance of getting low SSL by obese M was lower by 29% ($p=0.0014$), but in W increased by 19% ($p< 0.05$).

Conclusions: Depressive symptoms were observed more often in obese than in not obese persons, but low social support only in obese women. There were also the significant association between obesity and depressive symptoms and low social support level, both in men and women.

Memory Stick

EFFECTS OF NIACIN ON THE INCIDENCE OF NEW ONSET DIABETES AND CARDIOVASCULAR EVENTS IN PATIENTS WITH NORMAL AND IMPAIRED GLUCOSE

D. Maccubbin[1], **Vasilisa Sazonov**[2], C. Mccrary Sisk[1], P. Canner[3]

[1]Merck Research Laboratories, Rahway, [2]Merck Research Laboratories, Whitehouse Station, NJ, [3]Maryland Medical Research Institute, Baltimore, MD, USA

Objective: The Coronary Drug Project (CDP), a randomized, placebo-controlled clinical trial in men with previous myocardial infarction, showed that relative to placebo, niacin produced modestly favorable effects on cardiovascular outcomes after 5 years of treatment. Further analyses suggest that this effect is independent of baseline or on-treatment fasting plasma glucose (FPG) levels or the presence of metabolic syndrome. Nevertheless, because niacin raises glucose in some patients, there is reluctance to use it among impaired fasting glucose (IFG) patients who are at high risk of developing type 2 diabetes mellitus (T2DM). We conducted a *post-hoc* analysis from CDP to evaluate effects of niacin versus placebo on the incidence of new onset T2DM over 5 years in patients with normoglycemia or IFG.

Methods: New onset T2DM was defined by ≥1 of the following: clinical diagnosis of T2DM, use of an anti-hyperglycemic therapy, or 2 FPG values ≥126mg/dL. Normoglycemia and IFG are defined as FPG< 100mg/dl and FPG≥100 but < 126mg/dL, respectively.

Results: New onset T2DM was more prevalent among IFG (n=354 on niacin and 888 on placebo) versus normoglycemic (n=634 on niacin and 1560 on placebo) patients and was slightly higher with niacin versus placebo in both normoglycemic (6.8% vs. 4.9%; p=0.07) and IFG (19.8% vs. 15.2%; p=0.05) patients. Consistent with previous analyses, the cardiovascular benefit of niacin was independent of baseline glycemic status (normal, IFG, T2DM).

Conclusion: In spite of the increased risk of new onset T2DM with long-term niacin therapy, there is a potential cardiovascular benefit of niacin.

Memory Stick

ASSOCIATION OF USF1-S2 POLYMORPHISM WITH T2DM RISK IN THE TURKISH ADULT MALE POPULATION

Mehves Poda[1], E. Hörgüşlüoğlu-Güner[1], S. Humphries[2], A. Onat[3], G. Hergenç[4], G. Can[5], N. Erginel-Ünaltuna[1]

[1]Genetics, Istanbul University, Institute of Experimental Medicine, Istanbul, Turkey, [2]Center for Cardiovascular Genetics, Royal Free and University College London (UCL) Medical School, Rayne Institute, London, UK, [3]Emeritus, Department of Cardiology, Cerrahpasa Medical Faculty, Istanbul University, [4]Department of Biology, Yıldız Technical University, Istanbul, [5]Department of Public Health, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

Introduction: Upstream stimulatory factor 1 (USF1) belongs to the basic helix-loop-helix leucine zipper family. USF1 controls expression of genes involved in lipid and glucose homeostasis and colocalizes with familial combinedchyperlipidemia (FCHL) and T2DM.

Objectives: The aim of the study was to evaluate the association of USF1 (rs3737787 and rs2073658) polymorphisms with the type 2 diabetes (T2DM).

Methods: Participants, randomly selected from 59 residents of all 7 regions of Turkey, attended the survey in 2004, 2005, 2006 and 2007 years. Genotyping was performed using the Taqman technology. We genotyped 1976 subjects (51.4% female, mean age 54.1+/-11.6 years) and analyzed clinical data. All statistical analyses were performed using Windows SPSS version 14.0 software. The genotype data were tested for the HWE expectations.

Results: The genotype (rs3737787;GG:0.54, GA:0.38, AA:0.74; rs2073658; CC:0.54, CT:0.39, TT:0.068) and haplotype (CG: 0.7, CA: 0.038, TG: 0.034, TA: 0.228 at D'=0.824) frequencies were determined. An association was detected with the usf1s2 only. T2DM was more prevalent in males carriying the T (%11.2 *vs.* 15.9) allele of (p=0.036) rs2073658 polymorphism with regard to homogygotes for the C allele. After adjustment for confounding factors involving age, physical activity, BMI, smoking and alcohol consumption the logistic regression models revealed that, male carriers of the T allele of the rs2073658 polymorhysm (OR=1.813 [95%CI 1.20 -2.74], p=0.005) were under higher risk of developing T2DM. Such an association was not detected among females with either of the polymorphisms.

Conclusion: USF1-s2 polymorphism associated with the T2DM risk in the adult turkish male population.

Memory Stick

VASCULAR RISK FACTORS IN YOUNG PEOPLE: RESULTS FROM AN OBSERVATIONAL STUDY

Arturo Consoli[1], L. Zeppilli[1], M.T. Di Mascio[1], G. Carbotta[1], P. Lucchetti[1], G. Barilaro[1], D. Toni[1], M.L. Sacchetti[1], S. Vidale[2]

[1]Università di Roma, Sapienza, Roma, [2]A.O. S.Anna, Como, Italy

Introduction: Incidence, risk factors (RF) and etiology of stroke are quite different concerning young people (< 35 years). Although stroke is a leading cause of death and the first cause of disability in adulthood, few data describe this condition and RFs in young. Aim of the study was to evaluate vascular RFs and awareness about stroke in a young population.

Materials and methods: 818 undergraduates (mean age: , M/F: 1/1.4) underwent to a daily screening activity in Como and Rome Universities. Blood pressure, glycemia and anthropometric measures (weight, height and BMI) were recorded; main vascular RFs and awareness about stroke were evaluate by a standardized questionnaire. Statistical analysis was performed using chi-square test and analysis of variance (ANOVA).

Results: Smoke was the main RF (51.7%), overall in younger population (ANOVA; $p < 0.01$). Headache was prevalent in females ($p < 0.001$), while alcohol abuse in males ($p < 0.001$). 93.6% of people knew what is stroke, but only one third was aware about warning signs and emergency action at onset, in association with a previous family history of stroke ($p < 0.01$).

Conclusions: Main vascular RFs are quite present in young people. Smoke was the main RF in young females, while an excessive alcohol consumption in young males. These results underlines the need of preventive campaign in young people.

Memory Stick

MONOCYTE CHEMOATTRACTANT PROTEIN 1 (MCP-1): THE LINK BETWEEN OBESITY AND ATHEROSCLEROSIS?

Anna Abou-Raya[1], S. Abou-Raya[1], M. Helmii[2]

[1]Internal Medicine, Faculty of Medicine, University of Alexandria, [2]Biochemistry, Medical Research Institute, Alexandria, Egypt

Background: Obesity is associated with low grade chronic inflammation, a common feature of many of the obesity-related complications such as cardiovascular disease. Adipocytes secrete large amounts of proinflammatory mediators, particularly monocyte chemoattractant protein-1(MCP-1). MCP-1 is a chemokine that recruits monocytes into the developing atheroma and may contribute to atherosclerotic disease development and progression.

Objectives: To evaluate the association between MCP-1 and the risk for subclinical atherosclerosis in obese individuals.

Methods: Sixty obese normotensive adults, mean age 48.5 years and 60 age and sex and traditional risk factors matched adults of normal weight serving as controls were recruited. Serum levels of MCP-1, IL-6, TNF-alpha and high-sensitivity C-reactive protein (hs-CRP) were measured and correlated with traditional cardiovascular risk factors. Carotid intima media thickness (CIMT) was assessed by Doppler examination and the presence of plaques documented.

Results: Obese subjects had significantly higher levels of MCP-1 than controls, $p< 0.005$. Higher levels of hsCRP, IL-6 and TNF-alpha were found in obese individuals compared to controls. Obese subjects had a higher prevalence of subclinical atherosclerosis, 30% demonstrated subclinical atherosclerosis. CIMT was significantly higher in obese compared to normal weight individuals. MCP-1 levels were associated with age, family history of premature coronary disease, smoking, diabetes mellitus, hypercholesterolaemia, hs-CRP, IL-6, TNF-alpha and CIMT.

Conclusions: MCP-1 levels are increased in obesity contributing to systemic inflammation and subclinical atherosclerosis. These findings support the potential role of MCP-1 in atherosclerosis and cardiovascular disease. The implication of these findings is that MCP-1 is a potential biomarker target for drug development.

Memory Stick

C-REACTIVE PROTEIN BUT NOT SOLUBLE CD40 LIGAND IS ASSOCIATED WITH THE SEVERITY OF ANGIOGRAPHICALLY VERIFIED CORONARY ARTERY DISEASE

Vesna Spasojevic-Kalimanovska[1], S. Veljic[2], J. Kotur-Stevuljevic[1], N. Bogavac-Stanojevic[1], L. Memon[3], D. Kalimanovska-Ostric[4]

[1]Institute of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, [2]Biochemical Laboratory, Health Centre Berane, Berane, Montenegro, [3]Clinical Centre Bezanijska Kosa, [4]Clinical Centre Serbia, Belgrade, Serbia

Aims: One third to one half of the variation in vascular disease occurrence remains unexplained by traditional risk factors. Since atherosclerosis may, in part, be an inflammatory disease, circulating factors related to inflammation may be predictors of cardiovascular disease. The aim of our study was to assess the association of inflammatory markers with the occurrence and severity of CAD.

Methods: Serum levels of sCD40L and high-sensitive C-reactive protein (hsCRP) were measured in 127 CAD patients and 45 CAD-free subjects. Serum hsCRP was measured by an immunoturbidimetry assay and sCD40L by the ELISA test. According to the number of significantly stenosed (>50% of luminal diameter) vessels, all CAD patients were classified in four groups: without stenosis (0-vessel disease), 1,2 and 3-vessel disease.

Results: Median hsCRP levels were higher in CAD patients [3.75 (2.06-11.13) mg/L] than in CAD free [1.40 (0.71-2.88) mg/L] ($p < 0.001$). Median sCD40L levels were higher for CAD-free patients [511.98 (347.92-799.01) pg/mL] compared to CAD patients [612 (420.51-1000.00) pg/mL], ($p < 0.05$) CAD. The concentrations of hsCRP significantly increased with the severity of CAD defined by the number of stenosed vessels while the concentrations of sCD40L significantly decreased (Kruskal-Wallis test, $P < 0.05$). We performed an ordinal regression analysis to find proportional odds for each category of CAD extent. Only hsCRP was a significant predictor of the CAD extent.

Conclusions: hsCRP and sCD40L levels were significantly different in patients with CAD but only the level of hsCRP was an independent predictor of the CAD severity.

Memory Stick

FEATURES OF METABOLIC SYNDROME IN DEPRESSIVE DISORDER

Tomáš Vařeka[1], M. Zeman[1], M. Vecka[1], M. Dušejovská[1], R. Jirák[2], A. Žák[1], E. Tvrzická[1], J. Macášek[1], B. Staňková[1]

[1]IVth Dept Int Med, [2]1st Psychiatric Dept, 1st Medical Faculty, Charles University, Prague, Czech Republic

Background: Depressive disorder is considered to be a risk factor for the coronary heart disease and it is associated with increased risk for incident Type 2 diabetes as well. In some studies of the last years, it was suggested that DD may be associated with insulin resistance.

Materials and methods: In pilot study, the indices of lipid metabolism and glucose homeostasis were examined in group of 42 (7M/35F) patients with DD, in 57 probands (35M/22F) with metabolic syndrome (MetS) and in 49 (19M/30F) healthy persons. There were no significant differences in age, body mass index or share of smokers between investigated groups.

Results: We have found in the depressive patients, in comparison with controls, higher concentrations of serum insulin (12.53±10.73 vs.8.10 ± 3.06 μU/ml), C-peptide (0.87±0.34 vs. 0.67±1.20 pmol/l) (both P< 0.01), glucose (5.24±1.06 vs. 4.87±0.52 mmol/l, P< 0.05), higher HOMA index of insulin resistance (3.21±3.85 vs. 1.83±0.92, P < 0.001), triglyceridaemia (1.80±1.28 vs. 1.11±0.35 mmol/l, P < 0.001), concentrations of the conjugated dienes in LDL (62.9±24.4 vs. 47.4±12.9 μmol/l, P < 0.001) and microalbuminuria (P < 0.05). In comparison with MetS group, depressive patients were characterized by significantly lower triglyceridaemia (P < 0.001), plasma apolipoprotein B (P< 0.05), C-peptide (P < 0.01), uricaemia (P < 0.001) and by higher HDL-cholesterol (P < 0.001).

Conclusion: In presented pilot study, we have found the presence of some features of metabolic syndrome, especially insulin resistance and oxidative stress, in patients with DD.

Study was supported by the Research Project MSM 0021620820.

Memory Stick

CHARACTERISTICS OF PATIENTS WITH HIGH CLDL PLUS LOW CHDL AND/OR HIGH TG IN SPAIN. THE DYSLIPIDEMIA INTERNATIONAL STUDY (DYSIS-SPAIN)

Jesus Millan[1], J.R. Gonzalez-Juanatey[2], E. Alegria[3], C. Guijarro[4], J.V. Lozano[5], B. Gonzalez-Timon[6], G. Vitale[6]

[1]Hospital Gregorio Marañon, Madrid, [2]Hospital Clinico Univesitario de Santiago de Compostela, Santiago de Compostela, [3]Clinica Universitaria de Pamplona, Pamplona, [4]Hospital de Alcorcon, Alcorcon, [5]Primary Care Center La Serreria 2, Valencia, [6]Merck & Co., MSD, Madrid, Spain

Introduction: Low-HDL-C levels is a cardiovascular (CV) risk factor and high TG play also a role in CV disease (CVD). Patients with abnormalities in LDL-C, HDL-C and TG, have higher CV risk (CVR) than those with only high-LDL-C. In this study, we describe the characteristics of patients with combined lipid abnormalities and the prevalence of CVD.

Methods: Analysis of 3710 Spanish statin-treated patients in DYSIS (22063 partients in Europe and Canada). ATP-III recommendations were used to classify CVR, LDL-C goal and normal levels of HDL-C and TG.

Results: 3710 Spanish patients (35.7% with established CVD, 35% obese, 69% hypertensive, 39% with diabetes mellitus,18.6% active smokers) were analysed. LDL-C was not at goal in 50.4%. Among these, 46.2% had high-LDL-C alone, 29.7% normal-HDL-C and high-TG, as well as 15.8% low-HDL-C and high-TG. The prevalence of CVD was higher in patients with low-HDL-C (42.2% with 3 lipid parameters abnormal; 40.9% with LDL not at goal and low-HDL) compared to those with only high-LDL-C (35.9%, p=0.07 and 0.27 respectively). Stroke was also more frequent in patients with low-HDL-C (8%) and higher in those with low-HDL-C, high-TG and LDL-C levels (10.1%) comparing to patients that have only LDL-C not at goal (5.8%, p=0.32 and p< 0.05 respectively).

Conclusions: In this analysis of Spanish statin-treated patients,a large number of them showed multiple lipid abnormalities, not only abnormal LDL-C but also low-HDL-C and/or high-TG, especially those at higher CVR. The prevalence of CVD is more frequent in patients with LDL not at goal, low-HDL-C and high-TG levels. Treatment of the whole lipid profile may be of interest to reduce the risk of CV complications in statin-treated patients.

Memory Stick

CONSENSUS ON EARLY MANAGEMENT OF FAMILIAL HYPERCHOLESTEROLEMIA IN BELGIUM

Olivier Descamps, S. Tenoutasse, X. Stephenne, I. Gies, V. Beauloye, M.-C. Lebrethon, C. De Beaufort, J. Ducobu, M. Langlois, K. De Waele, V. Blaton, Y. Carpentier, G. De Backer, R. Rooman, A. Scheen, L. Van Gaal, E. Sokal, F.R. Heller

Centre de Recherche Médicale de Jolimont, Haine Saint-Paul, Belgium

A panel of Belgian paediatricians and adult specialists agreed on common attitudes regarding the management of FH children and adolescents, based on the review of the literature and AGREE methodology.

1 - Screening for FH should be performed only in children over 2 years if FH has been identified/suspected (by genetic tests or clinical criteria) in one parent.

2 - LDLC level above 135 mg/dl is very predictive for differentiating affected from non affected children. The genetic demonstration of a functional mutation provides an unequivocal diagnosis.

3 - A low saturated fat and low cholesterol diet may start after 2 years, under the supervision of a dietetician or nutritionist.

4 - The pharmacological treatment, using statin as first line, may start after 10 years in FH boys and after menarche in FH girls if LDL-C level remains above 190 mg/dL, or above 160 mg/dL in the presence of family history of early cardiovascular disease or with at least 2 other risk factors. The LDL-C targeting is 130 mg/dL, or 110 mg/dL if other risk factors are present.

Conclusions. Such a consensus may bring better homogeneity in identifying and treating FH in our countries. Because clear evidence are still lacking, we advocate for undertaking future collaborative work to examine the benefits of different strategies of management. In Belgium, there is also a need to design new conditions for the reimbursement of lipid-reducing drugs in children, as currently only a few may receive them.

Memory Stick

RELATIONSHIP OF INFLAMMATORY MARKER C-REACTIVE PROTEIN, LIPID MARKERS AND CONVENTIONAL CARDIOVASCULAR RISK FACTORS IN SUBJECTS WITH SUSPECTED CORONARY ARTERY DISEASE

Frank Beutner[1,2], D. Teupser[1], C. Tennert[1], M. Scholz[3], S. Gielen[2], M. Sandri[2], G. Schuler[2], J. Thiery[1]

[1]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, [2]Department of Cardiology, Heart Center, [3]Institute of Medical Informatics, Statistics and Epidemiolgy (IMISE), University Leipzig, Leipzig, Germany

Objective: The pathogenesis of coronary artery disease and other atherosclerotic diseases is strongly associated with serum lipid levels and an inflammatory process in the intimal vessel wall. The aim of this study was to investigate the relationship of inflammatory marker C-reactive protein (CRP) with serum lipid markers, other conventional risk factors and the angiographic extent of coronary atherosclerosis.

Methods: Blood was taken from 1514 patients before undergoing coronary angiography due to suspected coronary artery disease (Leipzig Heart Study). Apolipoprotein A, apolipoprotein B100, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and high-sensitivity CRP (hsCRP) were measured using commercial reagents and instruments (Roche Diagnostics), small-dense LDL was measured using a precipitation method (Denka Seiken).

Results: Female gender, increasing age, smoking, obesity and hyperglycemia were associated with raised hsCRP concentrations. CRP was inverse correlated with apolipoprotein A and HDL as well as weakly positive associated with apolipoprotein B100, but not with LDL, sdLDL and triglycerides. Higher CRP concentrations were associated with coronary artery disease. Multivariate analysis revealed HDL and accordingly apolipoprotein A, coronary artery disease and body-mass index as independent factors of the variance of CRP plasma concentration.

Conclusion: HDL but not LDL is associated with inflammatory marker CRP in subjects with and without coronary artery disease. Besides the known effects of HDL neutralizing C-reactive protein proinflammatory activity this study assumes a direct effect of HDL on CRP plasma concentrations.

Memory Stick

GENDER-DEPENDENT RISK ASSOCIATION OF UCP2-UCP3 LOCUS FOR CORONARY HEART DISEASE AND OBESITY AMONG TURKS

Mehves Poda[1], S. Humphries[2], A. Onat[3], E. Bayrak[1], G. Can[4], G. Hergenç[5], N. Erginel-Unaltuna[1]

[1]Genetics, Istanbul University, Institute of Experimental Medicine, Istanbul, Turkey, [2]Center for Cardiovascular Genetics, Royal Free and University College London (UCL) Medical School, Rayne Institute, London, UK, [3]Emeritus, Department of Cardiology, [4]Department of Public Health, Cerrahpasa Medical Faculty, Istanbul University, [5]Department of Biology, Yıldız Technical University, Istanbul, Istanbul, Turkey

Objectives: Study objective was to determine the possible contribution of UCP2-866G>A (rs6593366) and UCP3-55C>T (rs1800849) polymorphisms to CHD along with risk factors in the Turkish adult population.

Methods: Participants, randomly selected from 59 residents of all 7 regions of Turkey, attended the survey in 2004, 2005, 2006 and 2007 years. Genotyping was performed using the Taqman technology. We genotyped 965 males and 1,010 females (mean age=54,6 ±11,5) and analyzed data regarding clinical variables. All statistical analyses were performed using Windows SPSS version 14.0 software. Haplotypes were determined using the PHASE v2.1 software. The CubeX software was used for the computation of D´ and r^2 values of haplotypes, and the genotype data were tested for the HWE expectations.

Results: The rare allele frequencies were 0.25 for *UCP2* -866G>A (95% CI 0.24-0.26) and 0.22 for *UCP3* -55C>T (95% CI 0.20-0.23) in the Turkish population sample and fullfilled HWE expectations. The rs6593366-rs1800849 haplotype frequencies were determined (G-C: 0.61, A-C: 0.17, G-T: 0.14, A-T: 0.08 at D' 0.14). Obesity was more prevalent in males with the A-T haplotype (p=0.04), which largely persisted after adjustments. Logistic regression models revealed that, male carriers of the G-T haplotype had lower CHD risk compared with non-carriers (OR=0.56 [95%CI 0.33-0.98], p=0.041). Obesity risk was lower in G-T female carriers (OR=0.72 [95% CI 0.53-0.996], p=0.047).

Conclusion: The rs6593366-rs1800849 haplotype effect on CAD and obesity phenotypes in Turkish population is modulated by gender.

Memory Stick

FAMILIAL HYPOBETALIPOPROTEINEMIA - DETECTION BY USING PROGRAM OF PREVENTION OF CORONARY ARTERY DISEASE FROM CHILDHOOD IN THE CZECH REPUBLIC

Zuzana Urbanová[1], M. Šamánek[2]

[1]Dpt. of Pediatrics, 1st Faculty of Medicine, Charles University, [2]Kardiocentrum, University Hospital Motol, Prague, Czech Republic

Objective: Pediatric preventive program in the Czech Republic, which was started in the early 1990s, identifies children at high risk of atherosclerosis. Pediatricians are obliged to measure not only blood pressure and BMI, but also lipid profile (plasma total cholesterol, LDL, HDL cholesterol and triglyceride) in children with positive family history at the age of 5 and 13. This obligation is incorporated in the "Health Passport" of everyone born in the Czech Republic. The lipoprotein profiling is offered also to other members of the family. A high number of parents at high risk for coronary heart disease can be identified through their children. Children identified as being in increased risk of ischemic heart disease are referred to pediatric cardiologists or specialized centers to be followed up and treated.

Through this program we can detect also children with hypocholesterolemia (symptoms e.g. of malabsorbtion, celiac disease, cystic fibrosis, pancreatitis, ataxia, tumors, vitamin E deficiency etc.). Most subjects with hypocholesterolemia with clinical phenotype of heterozygous of familial hypobetalipoproteinemia are apparently asymptomatic and have been discovered during this screening. Few symptomatic cases of heterozygous have been reported. These subjects have gastrointestinal and neurological manifestations of variable severity and also fatty liver.

We report the clinical phenotype of family with familial hypobetalipoproteinemia.

Memory Stick

RELATIONSHIP BETWEEN HISTORY OF CARDIOVASCULAR DISEASES, DIABETES MELLITUS AND LIPID PROFILE IN TURKISH POPULATION WITH ACUTE CORONARY SYNDROME

Yuksel Dogan, Y. Doğan, K. Bayır, A. Soylu, G. Aşık, N. Yaşar, M. Taşkale, B. Kumbasar

DR. Sadi Konuk Research Hospital, Istanbul, Turkey

Background: Cardiovascular diseases are a consequence of genetic, environmental and atherosclerotic risk factors. It's still on debate which factors take place and how they affect the mortality in cardiovascular diseases.We investigated the relation between diabetes mellitus (DM), lipid profile and recurrence of cardiovascular accidents in patients with acute coronary syndrome in Turkish population.

Methods: 478 patients between 18-95 years of age were enrolled retrospectively. Their demographic properties and medical history were gathered from the reports. Patients with cardiovascular disease (CVD) history (including myocardial infarction, angina, cerebrovascular accident, peripheral arterial diseases) were classified as Group I and those without such history as Group II. Blood glucose levels and lipid profiles including total cholesterol (TC)/HDL and LDL/HDL ratios were recorded. Their mortality rates during hospitalization and after discharge upto 6 months were determined..

Results: 20.7% of cases were in Group I and 79.3% in Group II. Prehistory of CVD was higher between diabetics (28.7%) than nondiabetics (18.4%) ($p< 0.05$). HDL levels in Group I were lower than those in Group II ($p< 0.05$). LDL/HDL and TC/HDL ratio in males in Group I were higher than those in females ($p< 0.001$). Overall mortality rates were higher in Group I ($p< 0.001$).

Conclusion: It's found that DM, HDL, TC/HDL, LDL/HDL can be predictors for recurrence of cardiovascular diseases. Having a history of CVD and TC/HDL is determined to increase both in-hospital and 6 months mortality; and it can be asserted that diabetes can cause progression of cardiovascular diseases.

Memory Stick

HIGH-FAT, HIGH-FAT-PROTEIN DIETS AND EXERCISE: DISTINCT ADAPTATIONS IN THE THORACIC AORTA AND LIPID PROFILE

Cleverson Rodrigues Fernandes[1], K.M.A. Mata[1], D.S. Leonardi[2], F.T.T. Frajacomo[1], T. Marini[1], V.K. Cardoso[1], A.P. Farnesi[1], P. Paião[2], A.A.J. Junior[2], S.G. Ramos[1], S.B. Garcia[1]

[1]Department of Pathology, [2]Department of Medical Clinic, Ribeirão Preto Medical School, University of São Paulo (USP), Ribeirão Preto, Brazil

Introduction: One of the fashion diets would be that proposed by Dr. Atkins, and it consists of a diet having a liberated consumption of fats and proteins, and restriction for carbohydrates. The present investigation was conduced to assess the effect of exercise on Dr.Atkins diet-induced atherosclerosis lesions in Wistar rats.

Methodology: Sixty three-month-old male Wistar rats were randomly assigned to six groups: (G1) standard diet sedentary, (G2) standard diet aerobic training, (G3) high-fat diet sedentary, (G4) high-fat diet aerobic training, (G5) high-fat-protein diet sedentary, (G6) high-fat-protein diet aerobic training. Physical exercise consisted of swimming on a large aquarium. Rats swam 5 days a week, 60 minutes a day, for 8 weeks.

Results: Thoracic aortas were submitted a morphometric and histological analyze. Histological assessment demonstrated that atherosclerotic plaques were absent, however there were significant microscopic and biochemical differences among the groups, in the aorta as macrophages number (G4, G5), collagen (G4, G5) and enlargement of the aortic wall (G4,G5) were significant, although elastic fibers and nitrotyrosine formation no change. Biochemical analysis showed increase of triglycerides (G3,G4,G5,G6), total cholesterol (G4,G5), LDL (G5) but HDL no change.

Conclusion: Our results demonstrated a beneficial effect of exercise and specific adaptive responses in each nutritional intervention to exercise.

Memory Stick

FRAMINGHAM AND PROCAM SCORE PLUS CD44L SHOW STRONG ASSOCIATION OF PERIODONTAL DISEASE SCORE

Leopoldo Nucci da Silva[1,2], L. Timerman[3], J. Simone[2], C. Gun[3]

[1]Albert Einstein Israelita Hospital, [2]São Paulo University, [3]Dante Pazzanese Cardiology Institute, São Paulo, Brazil

Oral infection models have emerged as useful tools to study the hypothesis that infection and inflammatory reaction is a independent cardiovascular disease (CVD) risk factor. Periodontal infections are a leading culprit, with studies reporting associations between periodontal disease and CVD, but this studies the periodontal diagnosis and coronary risk show substantial variations. This study aimed to analysed the different methods of periodontal diagnosis (Periodontal Screening and Recording - PSR and Community Periodontal Index of Treatment Needs - CPITN) and correlation with Framingham and PROCAM coronary risk PLUS CD44L. The result shown strong and significant associations between periodontal diagnosis (r=0,830) and coronary risk (r=0723). Evidence continues to support an association among periodontal infections, atherosclerosis and vascular disease in different periodontal diagnosis and coronary risk stratification methods.

Memory Stick

MICROALBUMINURIA IN METABOLIC CENTRE OUTPATIENTS

Hana Rusnakova, H. Pittrova, D. Rajdl, J. Racek

Charles University Hospital and Medical Faculty in Pilsen, Pilsen, Czech Republic

Aim: To determine frequency of microalbuminuria in metabolic centre outpatients with one or more risk factors for cardiovascular diseases.

Metods: For the examination of microalbuminuria we colleced 2nd morning urine sample. We determined concentration of albumin by immunoturbidimetry (normal value 2.8 - 22.8mg / mmol creatinine). Data are expressed (if not stated otherwise) as median (interquartile range). For statistical comparisons we used Wilcoxon unpaired test.

Results: In the period October - November 2008, we measured microalbuminuria in 174 metabolic centre outpatients. 165 (95%) patients were treated hypolipidemic drugs, 124 (71%) patients were on antihypertensive therapy. 82 (47%) were patients with metabolic syndrome. Microalbuminuria was positive in 18 patients (10%); 11 (61%) of them had metabolic syndrome. The difference between microalbuminuria in patients with metabolic syndrome (0.7 [0.4-1.6] mg/mmol crea) and without metabolic syndrome (0.5 [0.3-1.0] mg/mmol crea) was statistically significant ($p < 0.05$ 95% CI 0.0001 - 1.3). Various other risk factors were: hypertension 68%, waist circuit (males ≥ 102cm, females ≥ 88cm) 48%, triglycerides (> 1.7 mmol/l) 48%, HDL cholesterol (< 1.0 mmol/l for males, 1.3 mmol/l for females) 15%, impaired glucose tolerance 22%, diabetes mellitus 10%.

Conclusion: Low incidence of microalbuminuria in metabolic centre outpatients is probably due to effective farmacotherapy and life-style intervention and certifies positive influence on risk factors of atherosclerosis.

Our plan for near future - to examine microalbuminuria in newly acquired outpatients without any therapy and compare them to patients with established therapy.

Memory Stick

ASSOCIATION BETWEEN CARDIOVASCULAR RISK FACTORS AND PRESENCE AND EXTENT OF CORONARY ATHEROSCLEROTIC PLAQUE AS DETECTED BY MSCT IN EGYPTIAN PATIENTS

Fatma Aboul-Enein

Cardiology, Alexandria University, Alexandria, Egypt

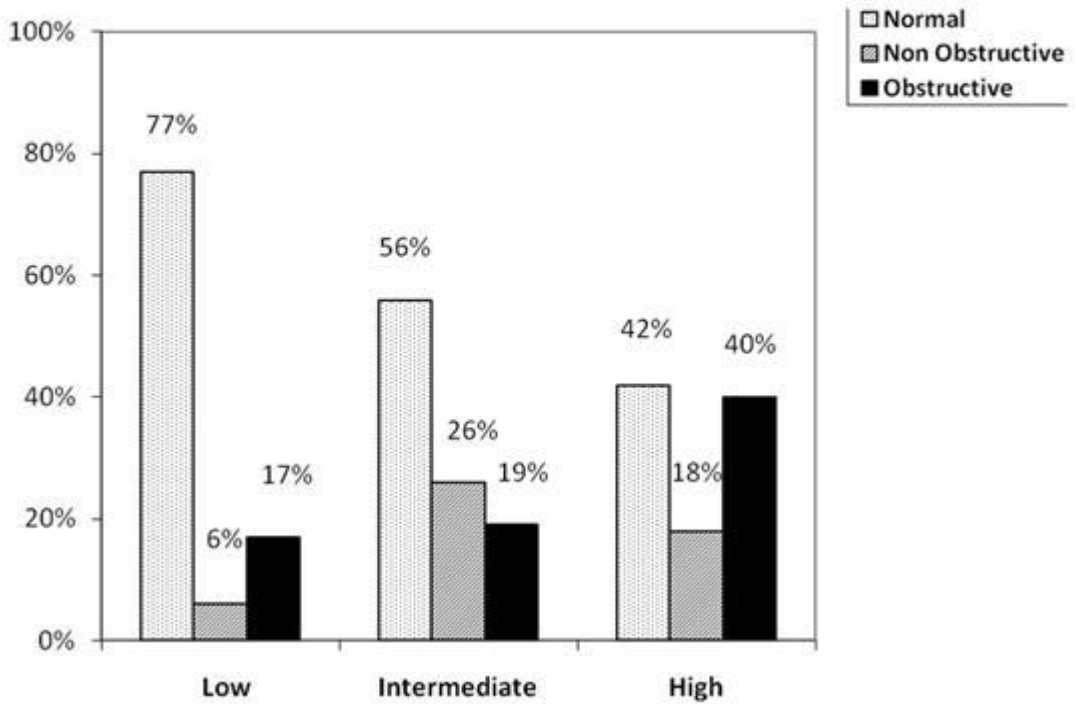
Framingham Risk Score (FRS) uses traditional risk factors (TRF) to categorize patients (pts) according to their risk for cardiovascular events and to establish treatment guidelines.

Aim: To investigate prevalence of coronary artery plaques (CAP) using computed tomography-based angiography (CTA) in Egyptian pts with no history of coronary artery disease (CAD) to evaluate whether TRF are related to prevalence of CAP.

Method: 1200 consecutive pts referred for CTA; pts with history of CAD were excluded, resulting in a cohort of 896. Age of 56.5±10.3 years, with 2.9 ±1.5 TRF and average FRS was 21.9±16.8. CTA was analyzed and pts without CAP were considered normal; an abnormal CTA is defined in the presence of ≥1 CAP. These were classified as having obstructive (≥50%) in 1 or more coronary arteries or non-obstructive (all < 50%).

Results: 43.8% (n=392) of the pts had CAP; 63.3% of which are obstructive. A total of 32.3 % (n=160/496) non high-risk FRS pts had CAP, reclassifying them to high-risk. 17.8% (n=88/496) of pts in non high-risk FRS had obstructive CAP. Among high-risk pts 42 % (n=168/400) had no CAP; reclassifying them as low risk. The prevalence of any CAP by the FRS category is shown in Figure 1.

Fig: Multislice Computed tomography coronary angiography MSCT results reclassify FRS categories



[Figure 1]

Conclusion: CTA reclassifies patients in both low and high FRS categories and may further influence clinical decision making.

Memory Stick

C-REACTIVE PROTEIN AND HEMOGLOBIN LEVELS PREDICT FUTURE ADVERSE CARDIAC EVENTS IN PATIENTS WITH CHRONIC STABLE ANGINA PECTORIS

Pablo Avanzas[1], R. Arroyo-Espliguero[2], A. Domínguez-Rodríguez[3], J.C. Kaski[4]

[1]Area del Corazón, Hospital Universitario Central de Asturias, Oviedo, [2]Cardiology, Hospital Universitario de Guadalajara, Guadalajara, [3]Cardiology, Hospital Universitario de Canarias, Tenerife, Spain, [4]Cardiovascular Biology Research Centre, St George's University, London, UK

Introduction: C-reactive protein (CRP) is an established marker of cardiovascular risk. Anemia is associated with worse symptoms and a significant increase in mortality in patients with advanced heart failure. The aim of the present study was to examine the prognostic significance of CRP and hemoglobin (Hb) levels in patients with chronic stable angina (CSA).

Methods: We carried out a 1-year follow-up prospective study in 215 CSA patients undergoing diagnostic coronary angiography. Coronary angiograms were scored according to Sullivan´s score, which includes vessel score, stenosis score and extension score. The primary study endpoint was the composite of non-fatal myocardial infarction, unstable angina and cardiac death. Hb and CRP levels were measured at study entry.

Results: 33 patients (15.3%) had adverse coronary events during follow-up. Patients with events had higher CRP levels (2.7 [1.4-5.25] vs 2.1 [1-4.7]; P=0.03) and lower Hb levels (13.5 [12.1-14.4] vs 14.3 [13.3-15]; P=0.002) compared with patients without events. After adjusting by confounders, multiple logistic regression analysis revealed that in addition to Hb levels below median (OR 2.7 [1.1 to 6.9]; P=0.03), CRP levels (0.04), severity of coronary artery disease (P=0.04) and a history of previous infarction (P=0.02) were independent predictors of future cardiac adverse events.

Conclusion: Hb levels predict the occurrence of adverse cardiovascular events in patients with CSA, supporting a role of anemia in the development of acute coronary events. Hb, CRP and CAD severity are independent predictors of risk in patients with CSA and provide complementary prognostic information.

Memory Stick

GENDER DIFFERENCES IN THE PREVALENCE OF CARDIOVASCULAR DISEASE RISK FACTORS AND CORRESPONDING 10-YEAR CARDIOVASCULAR RISK, AMONG SUBJECTS WITH METABOLIC SYNDROME

Theodoros Chimonas[1], V. Athyros[2], E. Ganotakis[3], V. Nicolaou[4], D. Panagiotakos[5], M. Elisaf[1]

[1]University of Ioannina, Ioannina, [2]Aristotle University of Thessaloniki, Thessaloniki, [3]University of Crete, Heralkion, [4]Red Cross Hospital, [5]Harokopio University, Athens, Greece

Purpose: Gender differences in the clustering of CVD risk factors among people with metabolic syndrome (MS) has rarely been investigated. Thus, we sought to evaluate CVD risk factor prevalence and estimated CVD risk in a Greek population.

Methods: A random sample of 824 male and 1199 female subjects with MS (NCEP ATPIII), but without diabetes mellitus or established CVD, was selected from several Greek areas. Several clinical and biochemical markers were measured. Ten-year risk estimates for fatal CVD were calculated.

Results: Women with the MS were older than men (57.6±10.3 vs 55.7±11.1 years, p< 0.001). Elevated BP levels and hypertriglyceridemia were more common in men than women (90.0 vs. 85.9% and 86.8 vs. 75.2%, respectively; p< 0.001), whereas low HDL-C and abdominal obesity were more common in women (59.1% vs. 65.1% and 83.6% vs. 97.0%, p< 0.001). The total number of metabolic criteria was equally distributed between the sexes, with 35.0% of men vs 32.7% of women showing any 3 criteria, 39.4 vs 39.9% any 4 criteria and 25.6 vs 27.4% exhibiting all 5 defining criteria. The 10-year risk for fatal CVD events was almost threefold higher in men (8.0±8.7% vs 3.0±3.8%, p< 0.001 using ESC SCORE and 8.6±8.1% vs 3.6±4.2%, p< 0.001 using Framingham model).

Conclusions: The MS is influenced by different factors in men and women, with men being at significantly higher risk for CVD. This information can be used for planning a better population-wide strategy for the prevention and treatment of the MetS in both sexes.

Memory Stick

PLASMA AMINOTHIOLS STATUS IN THE POPULATION OF THE ISLAND OF SÃO JORGE (THE AZORES' ARCHIPELAGO, PORTUGAL)

Rita Ferin[1,2], C. Correia[1], M. Raposo[2], B. Gonçalves[1], C. Gonçalves[3], J. Baptista[1,2], M.L. Pavão[1,2]

[1]DCTD, [2]CIRN, University of the Azores, Ponta Delgada, [3]Unidade de Saúde da Ilha de São Jorge, São Jorge, Portugal

Hcy, Cys, Cys-Gly and GSH are low molecular weight thiols that play important roles in the metabolism and homeostasis of the organism. An altered plasma aminothiols status can cause oxidative stress, thus contributing to atherogenesis. The aim of this study was to evaluate the plasma levels of total Hcy, Cys, Cys-Gly and GSH, as well as GGT activity in apparently healthy subjects from the Island of São Jorge, taking into consideration gender and lipid profile. The study group was formed by 73 subjects with no chronic diseases, aged 20 to 60 years, born living in the island of São Jorge. The evaluation of the four aminothiols was carried out by HPLC with an isocratic reverse-phase column using a fluorescence detector. Mean values of GSH (1.8±0.6µM) and Cys (240±35µM) content were under reference values, but Cys-Gly was above. Plasma total Hcy, Cys and Cys-Gly levels, as well as GGT activity were significantly higher in men than in women (respectively, 30%, 7%, 17% and 65%). About 63% of subjects were hyperlipidemic, mainly hypercholesterolemic. Cys concentration was increased by 9% in hyperlipidemics *vs.* normolipidemics. In subjects with altered thiol status men, but not women, revealed a moderate hyperhomocysteinemia. Also Cys-Gly concentrations were significantly higher in men than in women. In spite of being apparently healthy, all subjects (namely men) have depleted antioxidant defenses, indicating high oxidative stress. Alterations in plasma GSH, Cys-Gly and Cys concentrations, taken together, and independently of dyslipidemia, could be considered as early markers of atherosclerosis.

Memory Stick

LABORATORY EVALUATION OF HYPERURICEMIA AND STUDY OF DISEASE'S OUTCOME IN PATIENTS OF THE NORTH GREECE

Charalampos Charalampous, S. Patiakas

Microbiological Laboratory, General Hospital of Kastoria, Thessaloníki, Greece

Objective: The prevalence hyperuricemia and the patient's compliance to treatment and to dietary recommendations in patients from the Prefecture of Peonia in the Kilkis area in West Makedonia.

Methods: During the last 3years we performed measurements of the uric acid plasma levels of 3256 people and we found 187 having hyperuricemia. They were 139 men (74.3%) and 48 women (25.7%) with mean age 65.9±3.6, and 61.5±3.1. From them 146, (78%) had suffered in the past from acute single arthritis crisis. Even though diet was recommended to them, only 116 (62%) had a satisfactory compliance to the diet. Laboratory tests and appropriate follow up was performed with repeated measurements of disease's course based on the method of chemiluminencence.

Results: The first obtained measurements following the diet onset were 8.2±0.51 and following the allopurinole addition the values were 4.26±0.98. In contrast with this group the patients who showed no compliance to diet recommendations had: 7,96±1,22 mg and 5.81±1.45 after the allopurinole addition.

Conclusions:

1) We showed that the hyperuricemia is frequent in the healthy population especially to males so we recommend to test for uric acid plasma levels especially in males with non specific symptoms of arthritis.

2) The compliance to diet recommendations is equally important to medication treatment in those patients and should be emphasized both by laboratory and clinical physicians.

Memory Stick

INCIDENCE OF OBESITY OF THE YOUNG AND REGISTRATION OF THE PROGRESSION OF BMI OVER TIME

S. Patiakas[1], **Charalampos Charalampous**[2]

[1]Microbiological Laboratory, General Hospital of Kastoria, Kastoria, [2]Internal Medicine Clinic of the Psychiatric Hospital of Thessaloniki, Thessaloníki, Greece

Aim: Registration of incidence of obesity of the young and study of BMI in correlation to demographic and socioeconomic factors over time.

Material-methods: The study involved 465 young adults (218 men and 247 women) aged 18-29 years. Their anthropometric parameters were registered (height, BW, waist circumference etc), demographic data were recorded (occupational history, educational, social and economic status)and their biochemical and lipid panel were investigated (Glu, TG, HDL-chol, LDL-chol, total cholesterol etc.). The first registration was done in 1999 (164 subjects), the second in 2003 (155 subjects) and the third in 2007(146 subjects).

Results: In 1999 median BMI was 24,4 Kg /m^2, in 2003 24,9 Kg/m^2, in 2007 25,2 Kg/m^2. The incidence of overweight and obese according to WHO classification, was 26% and 8% respectively in 1999, 29% and 12% in 2003 and 31% and 13% in 2007.Hypercholesterolemia (200mg/dl) and hypertrigliceridemia (>150mg/dl) were diagnosed in 13,2% and 6,2% respectively in 1999 and in 14,8% and 7,6% in 2007. Although in the last two surveys a negative correlation between obesity and educational status came up, there was no relevant statistic correlation between obesity and place of residence or socio-economic status, in neither of the three clinical trials.

Conclusions: It is demonstrated that the last few years there is a significant progressive increase at BMI and a high incidence of overweight and obese young adults.The only parameter that influences the expansion of the problem of obesity is the educational status which emphasizes the need for continuous and intense preventing campaign.

Memory Stick

LIPIDEMIC PROFILE STUDY OF WOMEN AFTER LONG-TERM ADMINISTRATION OF CONTRACEPTIVE PILLS

Charalampos Charalampous[1], S. Patiakas[2], E. Pavlidou[3], E. Savvidou[3]

[1]Internal Medicine Clinic of the Psychiatric Hospital of Thessaloniki, Thessaloníki, [2]Microbiological Laboratory, [3]Gynecological Clinic, General Hospital of Kastoria, Kastoria, Greece

Aim: To study the effect of oral contraceptive pills on the lipids metabolism, given the fact that in literature and relevant studies, contradictory opinions are expressed.

Material - method: 53 women were studied, aged from 23 to 45, who used contraceptive pills for a period longer than 3 months. Their lipidemic profile was studied with the use of an automated biochemical - immunological analyzer. The results were compared to those of 60 healthy women, of similar age, who were the control group.

Results: Elevated levels of Lp(a) >30mg/dl were found in 12 women (22.65%) taking contraceptives, while in the control group only 4 women (6.7%) were found with elevated levels of Lp(a). The LDL-cholesterol and triglyceride average values were 141+/-39 mg/dl and 133+/-45mg/dl in the first group, and 127+/-36mg/dl and 130+/-44mg/dl in the control group. Further scrutiny revealed lower-limb deep venous thrombosis in 3 women taking oral contraceptive pills.

Conclusions: It is proven, that despite the fact that circulating contraceptive pills contain lower doses of estrogens, their long-term administration affects the metabolism of lipids, inducing an increase mainly in the levels of Lp(a), while the variations in LDL-cholesterol and triglyceride values seem to be smaller. Taking in to consideration the fact that Lp(a) is not only a risk factor for cardiovascular disease and vascular strokes, but also for many researchers an independent risk factor for thromboembolic episodes, a regular and thorough scrutiny of the lipidemic profile of women taking contraceptives for a long period of time is strongly recommended.

Memory Stick

INFLUENCE OF ATHEROSCLEROTIC DISEASE RISK FACTORS IN THE DEVELOPMENT OF ATRIAL FIBRILLATION IN THE POSTOPERATIVE PERIOD OF CARDIAC SURGERY

Renato J. Alves, B.C. Andrade, R.N. Campos, K. Nakiri

Santa Cruz Hospital, Sao Paulo, Brazil

Objective: To evaluate the association of atherosclerotic disease risk factors with the higher incidence of atrial fibrillation in the early postoperative period of elective cardiac surgery.

Methods: Retrospective study carried out in 140 patients, 97 (69%) males, mean age 62 years, submitted to cardiac surgery (coronary artery bypass grafting and/or valvular surgery). The patients were evaluated by continuous cardiac monitorization until the hospital discharge, correlating the main atherosclerotic disease risk factors (advanced age, diabetes, arterial hypertension, smoking and dyslipidemia) and the development of postoperative atrial fibrillation.

Results: Atrial fibrillation occurred in 23 (16%) patients in our population, more frequently occurring in the first day postoperative and in males than females, 19,8 versus 9,3% (p=0,2), respectively. Atrial fibrillation occurred in patients relating to atherosclerotic disease risk factors as: hypertensive and normotensive patients, 18% and 4% (p=0,02); advanced age over 65 and less than 65 years, 30% and 2% (p=0,01); smoking and no smoking, 21% and 11% (p=0,08); diabetes and no diabetes, 18% and 13% (p=0,43); dyslipidemia and no dyslipidemia, 16% and 13% (p=0,65), respectively.

Conclusion: This study demonstrated that arterial hypertension and advanced age were the atherosclerotic disease risk factors more significantly associated with atrial fibrillation in the early postoperative period of elective cardiac surgery.

Memory Stick

RISK FACTOR DIFFERENCES PREDISPOSING TO RECURRENCE AND/ OR DEATH IN YOUNG EARLY ONSET CORONARY ARTERY DISEASE PATIENTS

Amitesh Aggarwal, S. Dwivedi, M. Dev

Department of Medicine/ Preventive Cardiology, University College of Medical Sciences, University of Delhi & GTB Hospital, Delhi, India

Objectives: To evaluate differences in coronary risk factors predisposing to recurrence and/ or death in early onset coronary artery disease (CAD) patients.

Methods: Coronary risk factor profile of 158 young (≤ 45 years) patients with acute coronary syndrome was compared with 31 young (≤ 45 years) patients presenting with recurrent acute coronary syndrome and/ or death studied from Jan 2008 to Sep 2009 at UCMS-GTB Hospital.

Result: In young, compared to new acute coronary patients, patients presenting with recurrence and/ or death were older (41.9 ± 5.1 vs 38.4 ± 5.1 years), had greater prevalence of hypertension (32.3% vs 29.1%), diabetes mellitus (32.3% vs 30.4%), central obesity (54.8% vs 44.8%), family history of premature CAD (48.4% vs 43%), lower high density cholesterol (HDL-C) (76.2% vs 24.6%), increased low density cholesterol (LDL-C) (38.1% vs 31.7%), arcus juvenilis (51.6% vs 43.7%), premature balding (48.4% vs 41.8%) and carotid plaques (9.7% vs 7%), respectively. Mean carotid intima media thickness was also higher in group with recurrence and/ or death (0.66 ± 0.11 vs 0.63 ± 0.13 mm). Low HDL-C was statistically most significant risk factor associated with future risk of recurrence and/ or death.

Conclusion: Our data indicates that presence of low HDL-C, family history of premature CAD, hypertension, diabetes, central obesity, premature balding, arcus juvenilis and carotid plaques suggests future risk of recurrence and/ or death in young patients with acute coronary syndrome. These differences need to be further studied into in a lager sample over a longer period.

Memory Stick

POTENTIAL METABOLIC BENEFITS OF ADDING CHILLI PEPPER TO A MEAL

Madeleine Ball, I. Robertson, N. Harvey, K. Ahuja

School of Human Life Sciences, University of Tasmania, Launceston, TAS, Australia

Hyperinsulinaemia and lipid-peroxidation increase the risk of coronary heart disease. Some research indicates that consumption of chilli-containing meals reduces lipid-peroxidation. We studied the effect of chilli-containing meals on other metabolic parameters.

Objective(s): To investigate the effects of chilli-containing meals on postprandial glucose, insulin and c-peptide responses.

Design:

Study 1-Thirty-six healthy subjects consumed a standard bland meal and a meal containing 30g of MasterFood chilli-blend (55% cayenne chilli) after four-weeks on a bland (spice-free) diet.

Study 2- In a randomised cross-over study 17 participants consumed a bland meal, and meals containing 20g, 30g and 40g of chilli-blend. For both studies, plasma glucose, insulin and c-peptide were measured at regular intervals for two hours.

Results:

Study 1-The maximum increase in serum insulin and area under the curve (AUC) for insulin was 15% and 17% lower on the chilli meal compared to the bland meal (both $p=0.07$). AUC for glucose was also 10% lower after the chilli-containing meal than the bland meal ($p=0.065$). Although the c-peptide response was not different, the c-peptide/insulin quotient was significantly higher after the chilli meal than the bland meal ($p=0.045$).

Study 2- Increasing amounts of chilli-blend resulted in a dose-dependent decrease in AUC for insulin and c-peptide with the maximal postprandial increase 6-22% lower than after the bland meal (overall $p=0.04$). In both studies the chilli effect was greater in overweight subjects.

Conclusion: Chilli may reduce postprandial hyperinsulinemia without adverse effects on glucose, especially in overweight subjects, and hence have metabolic and cardiovascular benefits. This warrants further investigation.

Memory Stick

DETERMINATION OF CAROTID INTIMA MEDIA THICKNESS (CIMT) IN ASYMPTOMATIC BRAZILIANS

I.F. Espirito Santo[1], R.T. Nakamura[2], E.S. Parra[1], M.R.G. Danelon[1], **Eliana Cotta de Faria**[1]

[1]Department of Clinical Patology, [2]Radiology Department, University of Campinas, Campinas, Brazil

Aim: The measurement of carotid IMT is a well known, safe and simple method for detection of early atherosclerosis and reflects coronary atherosclerosis. We aimed at determining cIMT in a population sample of healthy Brazilian volunteers stratified by sex and age.

Methods: Ninety-eight clinically healthy volunteers, aged from 20 to 81y, 60% Females (F) and 40% Males (M) underwent high resolution β mode common carotid ultrasound, which was carried out by a single trained sonographer, blinded to the subjects' identities, using a 4-12 MHz linear array ultrasound imaging system. Plasma lipid profile and anthropometric measurements were performed. The cut-off values for age grouping were 45y F and 30y M (medians).

Results: The mean cIMT (mm) values found were: 0.653 M and 0.679 F (n= 98; $p \geq 0.05$) Comparing older men and women, the women had higher cIMT values (0.776mm F and 0.702, n= 30 vs 22; $p \leq 0.026$). Among younger individuals, the values were equal to 0.579, n=29 F and 0.589, n=17M. There were correlations between cholesterol and LDL-cholesterol with cIMT in the older subjects. cIMT correlated with BMI only in women. The frequency of atherosclerotic plaques in this study was equal to 14% F and to 5% M.

Conclusions: The cIMT values in this study, although safe according to the Brazilian Guidelines (< 1mm) and the European Guidelines (< 0.9mm), are 13% higher than the ones shown in the ARIC study indicating the need for cardiovascular disease prevention in Brazilians.

Memory Stick

STUDY RISK FACTORS OF CHRONIC NON-INFECTIOUS DISEASES BY A METHOD OF THE STATISTICAL ANALYSIS

Nina Nasonova, L. Shcherbakova

Institute of Internal Medicine SB RAMS, Novosibirsk, Russia

Introduction.: One of the biggest health hazards of the person is the growing burden of chronic non-infectious diseases (CNID). Comprehension of this threat has resulted the World health organization of public services in increase importance programs on preventive maintenance, the control and monitoring of distribution the CNID.

Material and methods: The data populations researches on N=8946 the persons, generated were used on the basis of representative sample (the period observed = 15 years). Upon termination of the period of supervision of the person, which had fatal event, were analyzed on structure of death rate. The analysis of distribution of frequencies of occurrence of diseases has confirmed the fact of the importance for region of the several basic CNID. Most frequently are cardiovascular diseases (720 person / 49,2 % from all cases of death / and cancer - 413 person / (28,2 %).

Results: The received results have allowed*:*

1. to specify the most significant for region CNID and to reveal factors - candidates for risk factors of concrete diseases for their monitoring and the further researches;
2. to prove suitability at an initial stage of application of typical statistical methods of processing of the mass data;
3. to raise the question about use known and development of the new diagnostic parameters which are taking into account joint influence on CNID of several risk factors;
4. to reduce factorial space up to *m=14* the risk factors subject to monitoring in the automated system of complex monitoring.

Memory Stick

THE RELEVANCE OF ANGIOTENSIN CONVERTING ENZYMEAND ENDOTHELIAL NITRIC OXIDE SYNTHASE GLU298ASP GENE POLYMORPHISMS FOR SUSCEPTIBILITY TO CORONARY ARTERY DISEASE

Anoosh Naghavi[1], S. Salimi[2], M. Firoozrai[3], S.M. Shafiee[3], A. Mohebbi[4]

[1]Cellular and Molecular Research Center, [2]Department of Biochemistry, School of Medicine, Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, [3]Department of Biochemistry, School of Medicine, [4]Department of Cardiology, Shahid Rajaee Heart Hospital, Iran University of Medical Sciences, Tehran, Iran

Introduction: There are several genetic and environmental factors which affect cardiovascular disease, among them, angiotensin converting enzyme (ACE) and endothelial nitric oxide (eNOS) gene polymorphisms are mostly interested to study in the case of genetic factors. In this study we compared the relevance of ACE insertion/deletion (I/D) gene polymorphism and eNOS Glu298Asp gene polymorphism for susceptibility to coronary artery disease (CAD) in Iranian population.

Patients and methods: 487 individuals including 224 patients with >50% angiographically established coronary stenosis from Shahid Rajaee heart hospital and 263 healthy subjects genotyped by a standard method. The systolic and diastolic blood pressure, serum cholesterol and LDL-C were significantly increased in CAD patients.

Results: The genotype frequencies of Glu298Asp polymorphism for Glu/Glu, Glu/Asp and Asp/Asp were 61.3, 32.2 and 6.5 percent in control subjects, and 46.5, 42.7 and 10.8 in CAD patients, respectively. The genotype frequencies differed significantly between the two groups (P=0.003). The frequencies of the Asp alleles were 32.2 and 22.6 percent for CAD patients and control subjects, respectively, and was different significantly between two groups (p= 0.001, odds ratio= 1.6).

Conclusions: Our results showed that there is no increased risk of CAD in association with DD genotype. Allele frequencies were also similar in both groups. Therefore, DD genotype does not increase the CAD susceptibility in the studied population. These results suggest that CAD is associated with Glu298Asp polymorphism of endothelial nitric oxide synthase gene as an independent risk factor of CAD, but ACE I/D polymorphism does not affect the susceptibility to CAD in Iranian population.

Memory Stick

IN APPLICABILITY OF THE CARDIOVASCULAR RISK PREDICTOR "SCORE" TO PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA (FH)

Olivier Descamps[1], J. Cooper[2], A. Neil[3], S. Humphries[2], on behalf od the Simon Broome Committee

[1]Centre de Recherche Médicale de Jolimont, Haine Saint-Paul, Belgium, [2]Cardiovascular Medicine, UCL Division of Medicine, [3]University of Oxford, London, UK

Introduction: Even if FH patients are considered at high cardiovascular (CV) risk, a precise risk stratification tool like "SCORE" in the general population may be interesting to evaluate the residual risk of treated FH patients. We evaluated how the usual SCORE ("UScore") and an extrapolation of SCORE to FH ("FHScore", based on the assumption that atherosclerosis induced before treatment has persistent negative effect) predict efficiently CV risk.

Methods: In 270 patients of the Simon Broome Register with full mortality data during their period of treatment, we compared the observed risk of CV mortality with the risk predicted by "UScore" and "FHScore". FHScore applies "SCORE" to the characteristics of the patients under treatment except that age is replaced by the number of years of treatment, plus, the age that would have a non FH patient with same CVD risk than the patient when he starts lipid-lowering treatment (estimated from published CVD mortality data).

Results: During the 9.4 years follow-up, 17 patients died; 9 from CVD deaths (= 3.4% of 10-years CVD mortality). The expected rates by UScore and FHscore were respectively 3.4% and 22% (expected number of deaths= 9.4 and 61). Although the UScore predicted the right number of death, these 9 dead patients were not amongst the patients with the predicted risk higher than 10%.

Conclusion: None of the examined prediction models are performing really well. The CVD risk of treated FH patients appears not influenced by the past exposure to very elevated levels of cholesterol.

Memory Stick

INFLAMMATORY STATUS AND OXIDATIVE STRESS BIOMARKERS IN NON-TREATED HYPERTENSION AND DISLIPIDEMIA

Corina Grigore[1], I. Stoian[2], O. Grigore[3], L. Dawkins[4], D. Isacoff[5], I.V. Bruckner[6]

[1]University of Medicine and Pharmacy Carol Davila, [2]Biochimie, University of Medicine and Pharmacy Carol Davila, [3]UPB, Bucharest, Romania, [4]The Wessex Institute for Health Research & Development, University of Southampton, Southampton, UK, [5]Internal Medicine-Coltea Clinical Hospital, [6]Cardiology-Coltea Clinical Hospital, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

Dislipidemia is one of the conditions encountered in clinical practice,that is why a closer attention at the related cardiovascular risk is neccessary. Oxidative modification of LDL play a central role in atherosclerosis. LDL-cholesterol susceptibility to oxidation is increased if it is associated with hypertrigliceridemia, smoking, hypertension, diabetes, low values of HDL-cholesterol.

Objectives: The purpose of this study was to investigate cardiovascular risk factors and oxidative stress parameters in a group of elderly patients (64.26±5.81 years vs. 65.33±3.47) with newly diagnosed hypertension or ignored treatment (TA 158.8±9/ 91.75±7.75mmHg) with/without metabolic syndrome (Mets).

Methods: Oxidative stress parameters were analyzed spectofotometric measuring the concentration of serum and erythrocyte superoxiddismutase, catalase and malonaldialdehyde and ceruloplasmin. To assess cardiovascular risk, we measured plasma levels of: triglycerides, total cholesterol, HDL-cholesterol, A1 and B-apoliproproteins, C-reactive protein, fibrinogen, ESR.

Results: Plasma levels of oxidative stress parameters determined in the elderly population with untreated essential hypertension,with/without Mets are significantly higher than control group ($p < .0001$, $\alpha = .05$). They also correlates strongly with the number of criteria for Mets, the lipid parameters and inflammatory status, they have an average correlation with the other cardiovascular risk factors considered and blood pressure. The coefficient of determination is significantly increased between the number of criteria for the Mets and oxidative stress parameters.

Conclusions: This study reveals the link between oxidative stress and Mets, and parameters of oxidative stress and lipid profile and inflammatory status, on the other hand. Should be elucidated whether oxidative stress is an independent factor in determining cardiovascular risk or is a consequence of disease evolution. Part of this study was financed by the national project ID-1472/2008: Evaluation of cronobiotics and antioxidant properties of light and melatonin. Place nontraditionali risk markers in cardiovascular risk assessment.

Memory Stick

RISK PROFILE IN PATIENTS UNDERGOING CAROTID ENDARTERECTOMY

Simone Vidale[1], S. Bellocchi[2], R. Caronno[3], M. Arnaboldi[1], A. Taborelli[2]

[1]Neurology Dept., [2]Neurosurgery Dept., [3]Vascular Surgery Dept., Sant'Anna Hospital, Como, Italy

Introduction: Several clinical trials have demonstrated the efficacy of carotid endarterectomy (CEA) in symptomatic and asymptomatic patients. Early and long-term positive outcomes depend on absence of adverse events (death, stroke, myocardial infarction) and patent carotid artery. High surgical risk patient is defined by the presence of several vascular risk factors (vRFs) or contralateral carotid thrombosis or female gender. Aim of the present study was to evaluate a possible relationship between comorbidities and short/long-term outcome.

Material and methods: Patients undergone CEA between June, 1st 2001 and December, 31th 2008 with complete data about vRFs, clinical features, lipid setting, and clinical and radiological follow-up. Primary outcome considered: death, vascular adverse events (stroke, transient ischemic attack, myocardial infarction) and significative restenosis (> 70%). For each patient a cumulative score was calculated. Statistical analysis was performed using chi-square test and analysis of variance (ANOVA).

Results: CEA was performed in n. 200 patients. We analysed 74 patients with complete and available data (M/F: 1/2.9; mean age: 69 ± 7.5 yrs). During follow-up 2 strokes and 6 restenosis occurred. A statistically significant association was observed between total score value and outcome ($p < 0.01$). No correlation was detected between single vascular risk factors and outcome. Low values of LDL cholesterol were related to a better outcome (ANOVA; $p < 0.01$).

Conclusion: In the present study patients with several and concomitant comorbidities have a higher risk of negative outcome. The relative low incidence of adverse events could be related to the selection of patients following current guidelines.

Memory Stick

ROLE OF SUSCEPTIBILITY GENETIC VARIANTS AND ENVIRONMENTAL FACTORS IN OBESITY RISK IN TWO SPANISH POPULATION BASED STUDIES

Felipe Javier Chaves[1,2], M.L. Mansego[1,3], S. Morcillo[4,5], G. Rojo[4,5], F. Martínez[3,6], J.C. Martín-Escudero[7], J. Redon[3,6], F. Soriguer[4,5]

[1]Genotyping Unit and Genetic Diagnostic, Fundación Investigación Clínico de Valencia, Instituto de Investigación Sanitaria INCLIVA, [2]CIBER de Diabetes y Enfermedades Metabólicas Asociadas "CIBERDEM" Valencia, [3]CIBER de Obesidad y Nutrición "CIBEROBN", Valencia, [4]CIBER de Diabetes y Enfermedades Metabólicas Asociadas "CIBERDEM" Málaga, [5]Endocrinology and Nutrition Service, Civil Hospital, Carlos Haya University Hospital, Málaga, [6]Hypertension Clinic, Hospital Clínico Universitario de Valencia, University of Valencia, Valencia, [7]Internal Medicine, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain

Blackground: In the last few years by using genome wide association studies several susceptibility variants for common obesity have been discovered. The most important ones are within the melanocortin-4 receptor (MC4R) and fat mass and obesity associated (FTO). The overall effect of these variants is very small and therefore the interaction with environmental factors, mainly energy intake, is necessary to develop obesity.

Objective: To investigate the role of these well known obesity susceptibility variants in two Spanish population based studies and their interaction with environmental factors.

Methods: 47 single nucleotide polymorphism in MC4R and FTO was evaluated in two Spanish general population: Hortega study (1502 subjects, mean age 54y, 49% women, 24% obese) and Pizarra study (989 subjects, mean age 46y, 62% women, 33% obese). General characteristics, clinical analytics and cardiovascular risk factors were recorded in both studies. Energy intake was assessed by mean of validated surveys. Genotyping was made with SNPlex. Subjects were divided into high or low energy intake according to the calories intake per day.

Results: In the initial analysis without considering environmental factors, we have been able to replicate the association with rs9939609 polymorphism in FTO in both populations. The study with selected polymorphisms taking into account energy intake grade, seems to increase the strength of the association but the analysis is still ongoing.

Conclusions: FTO gene plays an important role in obesity risk in Spanish population. The effect of some variants of this gene is modulated by individual energy intake in general population.

Memory Stick

FEMALE VERSUS MALE RISK FACTORS FOR ATHEROTHROMBOTIC DISEASE

William Feeman Jr

The Bowling Green Study, Bowling Green, OH, USA

Introduction: Atherothrombotic Disease (ATD) is the commonest cause of death for both men and women. ATD risk factors have been well studied in men, but not as well in women.

Objective: The objective is to demonstrate the ATD risk factors for women and to compare them to ATD risk factors in men.

Methodology: The ATD database (1974-2003) set up by the author from his private practice of family medicine is reviewed for the purpose of comparing ATD risk factors in women versus men.

Results: Women and maen have similar ATD risk factors, though they differ somewhat with regards to lipids, where women tend to have higher levels of LDL-cholesterol whereas men tend to have lower levels of HDL-cholesterol. This difference can be compensated for by using a ratio between LDL and HDL. Cigarette smoking is the chief risk factor, followed by dyslipidemia and hypertension. Diabetes mellitus is important, but mainly through cigarette smoking, dyslipidemia, and hypertension. These major ATD risk factors can be combined into a risk factor graph that is highly accurate in predicting ATD in both women and men.

Conclusion: The population at risk of ATD is predictable with high accuracy in both females and males, using simiar risk factors.

Memory Stick

THE FREQUENCY AND PREVELANCE OF THE CORONARY HEART DISEASE IN PATIENTS WITH METABOLIC SYNDROME

Huseyin Altug Cakmak, N. Karadag, B. Karadag, K. Cosansu, Z. Ongen, V.A. Vural

Cardiology, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey

Background: The objectives of this study was to investigate the frequency and extension of coronary artery disease (CAD) in patients with metabolic syndrome (MS) undergoing to coronary angiography.

Methods: 110 patients who underwent to coronary angiography (64 male, 46 female; mean age 50,5 ± 33.5) have been enrolled to the study. Three groups of patients are formed; group I composed of patients without MS and/or type II diabetes mellitus, group II composed of patients with MS and group III composed of patients with type II DM. Insulin resistance has been evaluated with HOMA-IR index. Coronary angiography was performed and number of vessels with significant stenosis (>50%) were documented.

Results: MS was found in 31 patients (28%), type II DM was present in 32 (29%) patients and 47 (43%) patients had neither DM nor MS. There was no difference in inflammatory markers as leukocyte count, CRP and fibrinogen levels between 3 groups of patients. Insulin resistance was %81 in MS group, %78 in type 2 DM and %57 in normal group. CAD incidence was significantly increased in patients with MS and Type II DM (control group %51, MS group %83, type 2 DM %84 (P=0,001) (Table 1) (Table 2).

	Group 1 Control		Group 2 MS		Group 3 DM		
	N	%	N	%	N	%	Chi –square p
CAG							
No Involvement	23	48,9	5	16,1	5	15,6	
Involvement	24	51,1	26	83,9	27	84,4	14,01 0,001

[Table 1]

	Group 1 Control		Group 2 MS		Group 3 DM		Chi-square p
	N	%	N	%	N	%	
CAG(number of vessels)							
0	23	48,9	5	16,1	5	15,6	
1	13	27,7	9	29,0	7	21,9	
2	7	14,9	10	32,3	6	18,8	
3	4	8,5	6	19,4	10	31,3	
4			1	3,2	4	12,5	25,11 0,001

[Table 2]

Conclusion: In conclusion, metabolic syndrome incidence was significantly high in patients undergoing to coronary angiography. Coronary artery disease incidence in patients with metabolic syndrome was as high as patients with DM type II. Metabolic syndrome was also found to be associated with the severity of coronary atherosclerosis.

Memory Stick

PREVALENCE AND RISK FACTORS FOR DIABETES: A TEN YEAR FOLLOW-UP STUDY OF THE YAEYAMA DISTRICT OF OKINAWA

Kyoko Okada, N. Furusyo, Y. Sawayama, Y. Kanamoto, M. Murata, J. Hayashi

Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan

Objective: To clarify the prevalence and incidence of undiagnosed diabetes and its relationship with various risk factors in the general population of Okinawa, 1,690 residents were screened in1989 and 1,163 in 1999.

Methods: Diabetes was diagnosed as both plasma glucose ≥126mg/dL (7.0mmol/L) and HbA1c ≥ 6.5%. Hyperglycemic subjects with fasting plasma glucose (FPG) 110mg/dL (6.1 mmol/L) below 126mg/dL were given a 75g oral glucose tolerance test (OGTT) and classified into a diabetic type, a borderline type, and a normal type.

Results: Of the residents surveyed in 1989, diabetes was found in 91 (5.4%), 61 (7.7%) men and 30 (3.3%) women, significantly higher for men than for women ($p< 0.05$). Residents with diabetes were significantly older, more likely to have significantly higher BMI, systolic blood pressure, a history of hypertension, serum triglyceride, ALT, and γ-GTP than non diabetic residents, both in 1989 and 1999. Over the ten years, the “consistently diabetic group” had significantly higher BMI ($p< 0.01$), and serum triglycerides ($p< 0.05$).

Conclusion: Our results from the Yaeyama dictrict of Okinawa showed that men had a higher prevalence of diabetes than women and that residents with diabetes had more obesity related disorders.

Memory Stick

GENES POLYMORPHISMS ANTIOXIDATIVE ENZYMES AND RISK CORONARY IN-STENT RESTENOSIS

A. Kaminnyi[1], J. Shuvalova[1], **Alexey Meshkov**[2], A. Samko[2], V. Kukharchuk[1]

[1]Department of Atherosclerosis, [2]State Institution Russian Cardiology Research Complex, Moscow, Russia

Aims: We examined the association of genes polymorphisms antioxidative enzymes (-262 C/T gene CAT, L55M and Q192R gene PON-1, Glu298Asp and -786T/C gene eNOS, Pro198Leu gene *GPx-1*, Ile105Val gene *GSTP*) with coronary in-stent restenosis and indices of oxidative stress.

Methods: The study included patients who underwent successful coronary bare-metal stent implantation and coronary angiography by 6 months. Genotype of genes polymorphisms was determined by polymerase chain reaction and restriction enzyme digestion. Plasma lipoperoxide and malondialdehyde levels and the catalase (CAT), glutathione peroxidase-1 (GPx-1) catalytic activity were measured before following coronary angiography.

Results: A total 101 patients were studied: the restenosis group (n=44) and no restenosis group (n=57). Carriers of the 298Asp allele of the *eNOS* Glu298Asp polymorphism showed a higher frequency of in-stent restenosis with an odds ratio of 2,79 (95%CI: 1,17-6,66) compared to 298Glu homozygotes. Carriers of the 198Leu allele of the *GPx-1* Pro198Leu polymorphism showed a higher frequency of in-stent restenosis with an odds ratio of 2,9 (95%CI: 1,23-6,84) and percent diameter stenosis (%DS) follow-up was higher by 21% (p = 0,01). Furthermore 198Leu allele of the *GPx-1* Pro198Leu polymorphism was associated with decrease of the GPx-1 activity (r = -0,4; p = 0,001) and increase lipoperoxide level (r = 0,43; p = 0,0002) and malondialdehyde level (r = 0,29; p = 0,014).

Conclusion: *eNOS* Glu298Asp polymorphism is associated with coronary in-stent restenosis. *GPx-1* Pro198Leu polymorphism is associated with frequency of in-stent restenosis and %DS follow-up against the background decrease of the GPx-1 activity.

Memory Stick

RISK OF CORONARY HEART DISEASE IN PATIENTS WITH CHRONIC RENAL FAILURE EVALUATION STUDY

S. Patiakas[1], **Charalampos Charalampous**[2], E. Basiliou[3]

[1]Microbiological Laboratory, General Hospital of Kastoria, Kastoria, [2]Internal Medicine Clinic, Psychiatric Hospital of Thessaloniki, Thessaloníki, [3]Nefrological Department, General Hospital of Kastoria, Kastoria, Greece

Aim: To study and evaluate the risk factors of coronary disease (CD) in patients treated with hemodialysis.

Material - method: A total of 56 Hemodialysis Unit patients were studied, 24 male and 32 female, with age average 62 years and with average time oh hemodialysis 6.8 years. Blood samples were taken, in order to determine the fasting blood sugar, the lipidemic profile, CRP, CK, CK-MB, creatinine (Cr) and troponin (T). At the same time, the blood pressure (BP) was measured, the smoking habits and family history of the patients were documented and their everyday exercise was evaluated.

Results: 18 patients (32%) presented blood sugar values>130 mg/dl, 8 patients (14%) had CRP>3 mg/dl, 14 (25%) had CK>150 U/l, 6 (11%) had CK-MB>30 U/l and 10 (18%) had T>0.06 ng/mL. As far as their lipidemic profile is concerned, 4 patients (7.1%) presented increased values of TRIG>200 mg/dl, 16 patients (28.6%) had TCHOL>240 mg/dl, 12 patients (21.4%) LDL-cholesterol>150 mg/dl, 4 male patients had HDL-cholesterol< 40 mg/dl and 4 female had HDL-cholesterol< 50 mg/dl (14.3% collective percentage). Hypertension was documented in 10 patients (17.9%), smoking was reported by 16 patients (28.6%), positive family history for heart disease was reported in 10 cases (17.9%) and limited everyday physical exercise was documented in 38 cases (67.9%).

Conclusions: It is proven, that in patients with chronic renal failure, the risk factors for coronary heart disease are higher compared to the general population. Therefore, they should be under constant and close monitoring, enabling an early diagnosis and treatment.

Memory Stick

OBESITY AND DISLIPIDEMIA

Gordana Grujic-Ilic[1], L. Pejcic[2]

[1]Pediatric, Health Center, [2]Pediatric Clinic, Nis, Serbia

Obesity is one of the risk factors of atherosclerosis in children and adolscents , followed by consequences which reach cardiovascular system (hypercholesterolemia, dislipidemia, hypertension)

The purpose of this study was to determine nutrition of children aged 2 to 19 , values of lipid and lipoproteins depending on nutrition, and percentage of children with values of lipid and lipoproteins which are high risk for coronary heart disease in adults.

This study included 1623 children. Each subjects height and body weight were measured, and body mass index (BMI) calculated ($BMI=TM/TV^2$)(kg/m^2). Overweight children were considered if body mass index was 25-29.9 kg/m2, and obese children if BMI was >30kg/m2 (Cool i sar.,2000). The recommendations N.Y.Ac.Sci. 1991.for values of lipid and lipoproteins which are high risk for coronary heart disease in adults were used.

Fasting plasma concentracions of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride were measured. Low-density lipoprotein cholesterol (LDL-C) non-HDL cholesterol (non-HDL cholesterol=TC-HDL), calculated.

Result: There were 11% obese and 17.9% overweight boys, and 7.41% and 15.58% girls.

Levels of TC, non-HDL-C, LDL-C, Tg, were significantly higher in obese boys (< 0.001) and obese girls than in nonobese children. The Levels of HDL-C were significantly lower (< 0.05: < 0.001)The percentage of lipid and lipoproteins which are high risk for coronary heart disease in adults were highest in obese boys., in overweight girls, only levels of Tg were highest in obese girls

Conclusion: The dates show the importance of early detecting and eliminating obesity in children for preventing consequences.

Memory Stick

COMPARATIVE STUDY OF RISK FACTORS IN YOUNG AND OLDER PATIENTS OF CORONARY ARTERY DISEASE IN A TERTIARY CARE HOSPITAL

Amitesh Aggarwal, P. Schroff, S. Dwivedi

Department of Medicine/ Preventive Cardiology, University College of Medical Sciences, University of Delhi & GTB Hospital, Delhi, India

Objectives: To study conventional coronary risk factors and compare them in young (age ≤ 45 years) and older (age > 45 years) patients of coronary artery disease (CAD) to understand their role in particular subgroup along with comparison of carotid intima media thickness (CIMT).

Methods: 20 consecutive patients of age ≤ 45 years (group I) and 40 consecutive patients of age > 45 years (group II) who suffered CAD for first time were recruited. Detailed history and examination including hypertension, central obesity, dyslipidemia, diabetes mellitus, smoking, family history of premature CAD and CIMT was made.

Results: Mean age was 39.6 ± 4.7 years in group I vs 61.2 ± 7.9 years in group II. Majority were males. Major risk factor were dyslipidemia and smoking (95% in group I vs 82.5% in group II and 70% in group I vs 60% in group II, respectively). 40% in group I and 50% in group II had central obesity. In group II it was found out that hypertensive patients were 4.3 times more likely to suffer from CAD. CIMT was significantly higher (0.07 ± 0.014 cms) in older patients compared to young (0.065 ± 0.015 cms). CIMT in younger group approached subclinical atherosclerosis suggesting subclinical atherosclerosis in the younger population.

Conclusions: Major burden of CAD can be explained by traditional risk factors only in both groups. Dyslipidemia and smoking were major risk factors and should be aggressively targeted. CIMT can prove to be useful noninvasive tool for early detection of subclinical atherosclerosis.

Memory Stick

ARE THE SAME RISK FACTORS INVOLVED IN CAROTID PLAQUE OCCURENCE AND SIGNIFICANT DOPPLER CAROTID STENOSIS ?

Belma Pojskic[1], A. Macic Dzankovic[2], D. Kolenda[3]

[1]Cantonal Hospital Zenica, Zenica, [2]Cardiology, General Hospital 'Abdulah Nakas', Sarajevo, [3]General Hospital 'Nova Bila', Nova Bila, Bosnia-Herzegovina

Introduction and aim: Risk factors which underlie atherosclerotic process are well known, but there is still a dispute which risk factor is more significant to development of various stages of process itself. The aim of this study is to identify risk factors significant for development of progressive atherosclerosis.

Methods and patients: Randomly selected 239 patients from data basis of carotid doppler examination, age 24-86, average 58. Parameters taken in consideration were age, sex, hypertension, diabetes, cholesterol, HDL, LDL , triglycerides, IMTCCA, carotid stenosis over 50% and plaque without Doppler measured stenosis.Statistic analysis was performed using SPSS 15.0 and STATA 9.

Results: There were 118 or 49, 4 % patients with hypertension and 74 or 31% diabetics. Patients with hypertension showed significant lower HDL, increased IMTCCA and plaque occurrence in comparison with non-hypertensive group. Diabetic patients had significant larger LDL, cholesterol, triglycerides, IMTCCA, lower HDL and higher incidence of plaque occurrence than non-diabetics. Sex was not significant for any of those parameters.

Plaque occurrence was significant in correlation with age, cholesterol, HDL, LDL, triglycerides and diabetes ($p< 0, 01$) and hypertension ($p< 0, 05$). Stenosis over 50% was connected with advanced age, cholesterol, LDL, triglycerides, IMTCCA ($p< 0, 01$); correlation with hypertension, diabetes and HDL was not significant.

Conclusion: Study has shown that plaque occurrence and stenosis over 50% correlated with advanced age, cholesterol, LDL, triglycerides, IMTCCA. Low HDL, diabetes and hypertension were important risk factors in atherosclerotic process, and correlated with plaque occurrence, but not with stenosis over 50%.

Memory Stick

EFFECTS OF APOE GENE POLYMORPHISM ON ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS IN MEN

Edita Stokic[1], I. Djan[2], M. Djan[3], D. Sakač[4]

[1]Department of Endocrinology, Clinical Center Novi Sad, [2]Department of Endocrinology, Clinical Center Vojvodina, [3]Faculty of Sciences, [4]Institute of Cardiovascular Disease, University of Novi Sad, Novi Sad, Serbia

The gene for ApoE is located on chromosome 19q13.2 and three common polymorphisms designated as e2, e3, and e4 code for the three major apoE protein isoforms (E2, E3, and E4). Genetic variation of ApoE is a major determinant of variation in susceptibility to dyslipidemia or coronary heart disease (CHD). In this research we investigated 60 men, 18 to 45 years old for the polymorphism of apoE gene. The study was conducted in a group of 60 male persons in whom, apart from anthropometric measures (weight, height, BMI, waist circumference, fat percentage), biochemical parameters were evaluated (total cholesterol, LDL-, HDL-cholesterol, glucose, insulin, fibrinogen and CRP). Forth exon of ApoE gene was amplified by PCR, and then digested with HhaI endonuclease. Fragments were separated by 4% agarose electrophoresis and genotypes were detected. In analyzed subjects 34 had genotype e3e3 and 17 had genotype e3e4. Rare genotypes were found in three men: e4e4, e2e4 and e2e3. Comparism of all anthropometric and biochemical parameters were performed between e3e3 and e3e4 genotype groups. It was shown that in e3e4 genotype group, following parameters were statistically higher: waist circumfence (105.5±8.8 vs. 99.1±9.9cm), BMI (29.7±3.2 kg/m^2 vs. 25.9±3.08 kg/m^2), insulin and HOMA IR (7.3±6.0 HOMA IR vs. 3.4±3.8). PCA analysis revealed factors dividing these two groups: waist circumfence, BMI, fat %, IA, insulin and HOMA IR. No difference was detected in cholesterol levels between those groups, but it was shown that larger number of analyzed subjects would reveal also a difference in LDL cholesterol.

Memory Stick

PECULIARITIES OF SYSTOLIC, DIASTOLIC, PULSE BP, HEART RATES AND FREQUENCY OF FATAL CARDIOVASCULAR DISEASE IN SIBERIAN POPULATION WITH METABOLIC SINDROM

G. Simonova, **Olga Glushanina**, Y. Nikitin, S. Malutina, L. Scherbakova

Institute of Internal Medicine SB RAMN, Novosibirsk, Russia

The analysis of different levels of blood pressure, heart rate and fatal cardiovascular disease (CVD) in a city Siberian population with metabolic syndrome (NCEP ATP III) (age group 45-69 years old, 2003-2005, from international projects HAPIEE).

Object: Representative samples of the Novosibirsk´s population for estimation of levels of systolic (SBP), diastolic (DBP), pulse BP (PBP), heart rate (HR) and frequency of fatal of CVD in groups with and without metabolic syndrome (MS) in 9329 subjects.

Methods: Population cross - sectionary study.

Results: Mean value of SBP-140,7; DBP- 88,4; PBP -52,2 mg/hg, HR - 79,7 in persons without MS and 154,1; 96,2; 58,0; 74,4 - in person with MS, appropriately, $p< 0,001$. SBP, DBP, PBP were significantly above in men without MS than in women. DBP, HR was prevalent in men with MS than in women, but PBP was significantly above in women. Frequency of SBP < 120 mg/hg was 9% in person with MS, but frequency of SBP 120-130 mg/hg was 15%. Frequency of fatal CVD in person with MS was 32%; among women with MS and levels of BP 140-160/90-99 mg/hg frequency of fatal CVD was significantly above than without MS.

The conclusion: These results show, that characteristics of SBP, DBP, PBP, HR prevalent in men and women with metabolic syndrome; this fact is negative factor growth of fatal of CVD in the Siberian population.

Memory Stick

GENDER DIFFERENCES AT ELDERLY PATIENTS WITH SUBCLINICAL ATHEROSCLEROSIS

Oana Vittos[1,2], F. Halici[1,3], I. Teodorescu[1]

[1]Medical Association 'Marie Curie', [2]Medcenter, Cardiology Dept, [3]Geriatrics, Medcenter, Bucharest, Romania

Aim: Subclinical atherosclerosis is increasing with age and is related to other cardiovascular and metabolic risk factors. All of these increase the prevalence of cardiovascular diseases in elderly people with major consequence on morbidity and mortality regardless of age.

Material and methods: 150 outpatients (age range 55-75 years) were admitted in our medical center (53,3% women and 46,6% men). They were globally evaluated and a comprehensive anamnesis was done. Clinical and laboratory evaluations were also done including electrocardiography, B-mode and Doppler ultrasonography and anthropometric tests were performed. Descriptive statistical data, independent t-test and a model of multiple regression were performed. P value less than 0.05 was considered significant (SPSS-10).

Results: Subclinical atherosclerosis was found at 37,55 % in women and in 28,55 % in men. A high prevalence of metabolic disease was diagnosed at both gender with a predominance in women (54%) . There were found positive correlations between body mass index, age and HDL-cholesterol ($p< 0.01$) and subclinical atherosclerosis in women and serum uric acid, glycemia, tryglicerides ($p< 0.05$) and subclinical atherosclerosis in men. Age, BMI and HDL-cholesterol were found as independent predictors for asymptomatic/subclinical atherosclerosis. Significant gender differences were noticed questioning about the overlap of gender role on cardiovascular risk factors and subclinical cardiovascular pathology.

Memory Stick

MOST IMPORTANT CARDIOVASCULAR RISK FACTORS IN PROGNOSIS OF PATIENTS WITH MIOCARDIAL INFARCTION-5 YEAR FOLLOW UP STUDY

Tohid Emami Meybodi[1], M. Emami[2], M. Hasan Lotfi[3], S. Mahdiye Namayande[3], S. Mahmood Sad[3]

[1]Cardiovascular Research Center, [2]Afshar Heart Center, [3]Yazd University of Medical Science, Yazd, Iran

Introduction: Cardiovascular diseases are increasingly recognized as a major cause of morbidity and mortality in almost all countries.myocard Infarction is usually accompanied by pain, increased enzyme values and ECG signs. Obviously, there are many distinct risk factors that can increase its incidence.

Objectives: To find out the survival rate and the most important predictors of death in our population.

Materials and methods: We followed 523 admitted MI patients (86% with first MI) for 5 years with a six-month follow-up interval.we collect all information needed Then we analyzed the data by SPSS software and with different statistical techniques such as Kaplan-Meier, Log rank test and Cox-proportional hazard ratio.

Results: cardiac death occurred in 132 patients (20%) in the univariation analysis many of the risk factors were significant. However, we inserted data in Cox - Proportional Hazard ratio analysis and Results revealed that some of the risk factors were meaningful.results show that; Risk of death in patients with positive familly history were 3 times more than others (HR=3.23,95%CI=1/17-8/91, P=0/023) and this Risk were 8 times more in those patients who had hypertension(stage 2) in comparison with rest of population (HR=8/44,95%CI=1/88-37/8, P=0/005).

Conclusions: Results showed that 20% of MI patients are at the risk of death and hypertension is the most important risk factor which can account for death after MI(as many of papers say it),so this is important to control hypertension as a main risk factor.

Keywords: MI-risk factors-follow up-death-hypertension

Memory Stick

WEEKLY AND SEASONAL VARIATIONS IN THE ONSET OF CARDIOVASCULAR EVENTS

Simone Vidale[1], R. Bonatti[2], M. Arnaboldi[1], C. Campana[2]

[1]Neurology Dept., [2]Cardiology Dept., Sant'Anna Hospital, Como, Italy

Introduction: The existence of a seasonal and weekly pattern of onset of cardiovascular events has been described for single disease. Aim of the study is to determine whether there is a similar variation in the occurrence of total cardiovascular accidents.

Materials and methods: All cases of patients with acute myocardial infarction (MI), ischemic (IS) and hemorrhagic stroke (ICH) and peripheral artery ischemia (PAI) between January, 1st 2005 and December, 31th 2006 were recorded. Included variables: age, date of onset (day of week; season), outcome at discharge (fatal or non-fatal events). Statistical analysis was performed using chi-square test and analysis of variance (ANOVA).

Results: Database included 2204 cases (ICH: 134; IS: 905; MI: 1045; PAI: 118). Patients with PAI were older than the others (ANOVA; $p < 0.001$). The highest number of cases occurred on Monday ($p < 0.05$), with a significative difference between ischemic and hemorrhagic events ($p < 0.05$). Incidence peak for ischemic events occurred in autumn and winter, while in summer for ICH ($p < 0.05$). Fatal events occurred mainly in older patients for each cardiovascular disease, PAI excluded ($p < 0.01$).

Conclusion: The results of this study confirm seasonal and weekly trends in cardiovascular events with a different pattern between ischemic and hemorrhagic accidents.

Memory Stick

TO ASSESS THE ATTITUDE AND NEGLIGENCE OF HYPERTENSIVE PATIENTS TOWARDS LIFESTYLE MODIFICATION

Sidra Jawed[1], S. Tai[1], M.A. Saleem[1], S. Bashir[1], M. Majid[1], A. Zaki[1], M. Kazmi[1], A. Qasim[2]

[1]Dow Medical College, [2]Cardiology, Civil Hospital Karachi, Karachi, Pakistan

Introduction: Hypertension is one of the most common human diseases prevalent worldwide. Mortality and morbidity due to hypertension has declined in the developed world but in developing countries like Pakistan, the problem is still grave. Life-style modification complementary to medical treatment has been proved to be the most effective means of controlling hypertension and its progression. Data regarding lifestyle modification in our part of the world is deficient.

Aims: To explore the attitude and assess negligence of hypertensive patients towards lifestyle modification.

Methods: Cross-sectional multi-centre analysis involving reputed government and private hospitals of Karachi. Data collected by convenience purposive sampling. Sample size is set at 300. Questionnaire- based interview of admitted patients as well as patients visiting the out-patient clinics, who are diagnosed to be hypertensive.

Results: 100 patients were interviewed: mean age of the population was 52 ±3 years; with equal no. of males and females. 52% were uneducated having a mean blood pressure of 143/91, 78% were taking anti-hypertensive medication regularly. 92% of total started dietary modification out of which 98% were counseled by doctors while only 2% by their families'. 36% started exercising out of which 60% were counseled about it. 66% reduced their intake of salt,10% stopped it completely. Majority of the patients who did not start dietary modification developed complications compared to only 30 patients who developed complication even after the modification.

Conclusion: As the study is ongoing, it is early to articulate about the negligent attitude of hypertensive patients towards their lifestyle.

Memory Stick

RISK FACTORS CONTROL BENEFITS IN A CARDIOVASCULAR UNIT

Ricardo Bagina, C. Baeta

Internal Medicina, HDJMG - ULSNA, epe, Portalegre, Portugal

Object: Analyze the impact of medical therapeutic and intervention cardiology in a high cardiovascular risk population followed in the Cardiovascular Risk Consulting Unit.

Method: We compared the patients in Primary (G1) and Secondary (G2) Prevention and those who had AMI (G2a).

Results: We studied a sample of 176 patients (M:78,9%, Age: 66,5 years; W:21,02%, Age: 69 years), divided in:

G1: 63 patients; 64,4 years; AHT-90%; DM2-68%; Dyslipidemia-59%; ATs-140,7mmHg; ATd-81,1mmHg; BMI-28,5; AP-100,1cm; HbA1c-7,6%; TC-173,5mg%; LDLc-96,1mg%; Triglycerides-151,5mg%; Anti-Agregants-71,43%; ACEI/ARB-90,48%; B.Blocker-16%; Statins-79%; Angioplasty-5%; Stent-2%; CABG-2%; CVD-Risk- 14% (Guidelines: 12%);

G2: 113 patients; 68,5 years; AHT-73%; DM2-41%; Dyslipidemia-38%; ATs-137mmHg; ATd-76mmHg; BMI-27,8; AP-100,3cm; HbA1c-7,3%; TC-162,9mg%; LDLc-91,3mg%; Triglycerides-140,4mg%; Anti-Agregants-97,4%; ACEI/ARB-85,8%; B.Blocker-54%; Statins-89%; Angioplasty-58%; Stent-57%; CABG-27%; CVD-Risk-18% (Guidelines: 16%);

G2a: 71 patients; 69,4 years; AHT-66%; DM2-34%; Dyslipidemia-38%; ATs-136,4mmHg; ATd-75,9mmHg; BMI-27,3; AP-99cm; HbA1c-7,1%; TC-161,4mg%; LDLc-91,0mg%; Triglycerides-134,9mg%; Anti-Agregants-100%; ACEI/ARB-88,73%; B.Blocker-56%; Statins-90%; Angioplasty-61%; Stent-61%; CABG-24%; CVD-Risk- 18% (Guidelines: 16%);

Conclusions:

1. Primary prevention patients:

- Present a worse metabolic control

- Are younger and with more controllable risk factors

- Less medicated with anti-agregants and b.blockers and had less interventional cardiology procedures

2. Secondary prevention patients:

- The patient's characteristics, with or without AMI, are similar

- Medical treatment and interventional cardiology are also similar

3. Risk Factor control's benefits:

- 1/3 risk's reduction in primary prevention

- Without additional benefit in secondary prevention

Memory Stick

RISK FACTORS OF IRANIAN PATIENTS WITH 3 VESSELS DISEASE CANDIDATE FOR CORONARY ARTERY BYPASS GRAFT SURGERY (CABG)

Majid Najafi Kalyani[1], N. Jamshidi[2]

[1]Fasa University of Medical Sciences, Fasa, [2]Kerman University of Medical Sciences, Kerman, Iran

Objective: Cardiovascular disease is one of the most common health problems that threaten life. Coronary artery disease is major cause of morbidity and mortality in human communities. CAD has many mental, physical and social consequences for sufferer. The aim of this study was to determine the modifiable risk factors among patient with 3 vessels disease (3VD) candidate for CABG.

Methods: This study investigated 1000 coronary patient with 3VD (660 males and 340 females) with mean age 54.8±9.01 years. Outcome variable included demographic and coronary risk factors parameters in patients referred to Jamaran heart hospital were collected. We used of a questionnaire for collecting data from patient and medical document. Using descriptive statistic performed data analysis.

Results: 66% of patients were male and 34% were female. Mean age of patients´ were54.8±9.01 years. History of diabetes, Hypertension and smoking was positive in 38%, 32% and 40%, respectively.11% had BMI≥30, %45.6 of patient had O+ (blood group). 87% of patient had total cholestrol≥200, 81% had TG≥200 and about 70% had LDL>100. There was statistically significant between sex with cigarette smoking, HTN, DM ($P< 0.05$) and age with TG ($P< 0.05$).

Conclusions: Results of this study showed that hyperlipidemia, diabetes and smoking are most common modifiable risk factors of CAD in 3VD. Findings of this study provide more information on the database of the risk factors among patients with progressive coronary artery disease in Iran. Therefore, applying education for changing lifestyle including cease smoking, controlling diabetes, healthy diet and exercise is necessary.

Memory Stick

PLANTS ARE POPULARLY USED BY ALTERNATIVE MEDICINAL PRACTITIONERS OF KISHOREGANJ DISTRICT, BANGLADESH TO TREAT VARIOUS BODY PAINS INCLUDING RHEUMATOID ARTHRITIS

Ariful Haque Mollik

Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Peoples Integrated Alliance, Dhaka, Bangladesh

Various types of body pains are common afflictions affecting people throughout the world. A more debilitating type of pain arises from rheumatoid arthritis, which is believed to affect at least 3% of the world's population, mostly the elderly. Since regular use or over-use of various pain-killer drugs may have side-effects, an alternative route to treat pain is through use of plants provided by alternative medicinal practitioners and which are generally believed to be without any side-effects. We accordingly conducted an ethnomedicinal survey amongst the alternative medicinal practitioners of Kishoreganj district, Bangladesh to gather information on plants used to treat various types of pain. Plants as pointed out by the alternative medicinal practitioners were collected and identified at the Bangladesh National Herbarium. Plants used to treat general body pains included *Datura metel*, *Achyranthes aspera*, *Ricinus communis*, *Croton bonplandianum*, *Piper betle*, *Lygodium flexuosum*, *Cyperus rotundus*, *Tagetes erecta*, *Bambusa arundinacea*, *Momordica charantia*, *Cinnamomum camphora*, *Pongamia pinnata*, *Azadirachta indica*, *Brassica napus*, *Aphanamixis polystachya*, *Linum usitatissimum*, *Cereus grandiflorus*, *Nigella sativa*, *Cynodon dactylon*, *Trewia nudiflora*, and *Polygonum persicaria*. Plants used to treat rheumatic pain included *Chenopodium ambrosioides*, *Withania somnifera*, *Cocos nucifera*, *Vitex negundo*, *Cissus quadranglaris*, *Grewia paniculata*, *Musa sapientum*, and *Eupatorium odoratum*. *Phyllanthus reticulatus*, *Eclipta alba*, *Curcuma longa*, and *Zingiber officinale* were used to treat stomach pains, *Coccinia cordifolia*, *Argyreia speciosa, Nicotiana tobacum*, and *Lawsonia inermis* while were used to treat headaches. Taken together, the above plants provide effective remedies for the rural population such that they do not have to visit modern medicinal practitioners.

Memory Stick

ASSOCIATION OF HIGH PCR LEVELS AND DYSLIPIDEMIA IN PATIENTS WITH ATRIAL FIBRILLATION

José Antonio Díaz-Peromingo[1], J. Grandes-Ibáñez[2], F. García-Suárez[1], J. Sánchez-Leira[1], M.D.C. Gayol-Fernández[1], J. Saborido-Froján[1], S. Molinos-Castro[1], P. Pesqueira-Fontán[1], M. Iglesias-Gallego[1], E. Padín-Paz[1], J. Naveiro-Soneira[1]

[1]Hospital da Barbanza, Riveira, [2]Complexo Hospitalario Universitario, Vigo, Spain

Introduction: Atrial fibrillation (AF) is the leading cause of embolic events in the adult population. The association of dyslipidemia leads to an increase in the atherothrombotic profile in these patients. The influence of inflammation has been not clearly assessed in these patients.

Methods: 137 patients with AF were included. Age, sex, known dyslipidemia, total cholesterol (TC), LDL, HDL, TG, Lp (a), and CRP levels were reported. Statistical analysis was made using a correlation analysis and the T-Student when needed.

Results: 79 patients were women and 58 men. Middle age was 72 (37-86). Known dyslipidemia was reported in 83 (60.58%) patients. In the dyslipidemic group, mean TC was 213 ± 94.7 mg/dL, LDL 152 ± 53.1 mg/dL, HDL 41 ± 8.4 mg/dL, TG 194 ± 94.6 mg/dL, Lp (a) 5.9 ± 2.8 mg/dL, and CRP 1.5 ± 0.9 mg/L. In the non-dyslipidemic group, mean TC was 153 ± 63.8 mg/dL, LDL 98.4 ± 37.2 mg/dL, HDL 48.2 ± 13.4 mg/dL, TG 116 ± 52.1 mg/dL, Lp (a) 4.2 ± 3.1 mg/dL, and CRP 2.7 ± 1.6 mg/L. Differences in TC, LDL and CRP levels between groups were statistically significant. CRP levels correlated inversely with the presence of known dyslipidemia.

Conclusions:

1.- Patients with AF are predominantly elderly women with known dyslipidemia.

2.- The presence of AF and dyslipidemia were associated with lower levels of CRP.

3.- CRP levels seem not be an useful test when assessing vascular risk in patients with AF associated dyslipidemia.

Memory Stick

USING BIOIMPEDANCE ANALYSIS FOR QUANTIFITATION OF BODY FAT AS RISK OF METABOLIC SYNDROM

Pavol Hlubik[1], H. Stritecka[1], J. Hlubik[2]

[1]Military Hygiene, University of Defence, Hradec Kralove, [2]Gerstner Laboratory, Czech Technical University, Prague, Czech Republic

Obesity with body fat accumulation in sub cutis part of human body presents risk metabolic syndrome development. Overweight and obesity are characterized by Body Mass Index (BMI) rising up using standard. % of body fat is next important characterization, which is needed to adjust on gender. For body fat measuring can be use many type of analysis, including body´s impedance analysis (BIA). Bio-impedance analysis - evaluation of body composition is important for relationship assessment between particular body segments (fat-mass FM, fat-free-mass FFM, total body water TBW, intracellular water ICW, extracellular water ECW).

The aim of work was evaluated change of TCHOL, LDL, TAG, HDL, body weight, BMI, % FM, FFM, visceral fat, ECT, ICT and waist. For BIA analysis was used InBody 720 in patients on low caloric diet as therapeutically regime for risk of metabolic syndrome decreasing. The full-scale body components analysis was done and the data from InBody 720 was statistically analyzed.

BIA is posing as important methods for change in fat distribution and mount diagnostic.

Supported by MO FVZ 0000502

Memory Stick

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) VAL66MET POLYMORPHISM IS NOT ASSOCIATED WITH MYOCARDIAL INFARCTION IN CZECH POPULATION

Jana Petrkova[1,2], A. Stahelova[2], N. Motakova[2], F. Mrazek[2], M. Petrek[2,3]

[1]Internal Medicine I, Faculty Hospital Olomouc, [2]Immunology, Palacky University, [3]Clinical Biochemistry and Immunogenetics, Faculty Hospital Olomouc, Olomouc, Czech Republic

Background: Brain-derived neurotrophic factor (BDNF) has been implicated in the pathophysiology of coronary artery disease (CAD). Recently, human BDNF Val66Met polymorphism has been associated with coronary artery disease in Chinese population (1). To explore further a possible role of this polymorphism as a genetic modifier in CAD we have investigated its association with myocardial infarction in Czech population.

Methods: BDNF Val66Met polymorphism was genotyped by real-time PCR in 180 healthy unrelated control subjects and in 217 patients with myocardial infarction diagnosed according to the international (ESC) consensus.

Results: The genotype and allele frequencies of the BDNF Val66Met polymorphism did not differ between patients and control subjects ($p > 0.05$). Two investigated groups also did not differ in carriage rates (phenotype frequencies) of BDNF Val66Met polymorphism.

Conclusion: The BDNF Val66Met polymorphism is not associated with myocardial infarction in Czech population. We could not, therefore, replicate the observation from China (1), which suggested that BDNF Met/Met genotype is a genetic modifier in CAD. Investigations in further centres and/or populations (2) are, therefore, necessary to obtain more information on possible role of Brain-derived neurotrophic factor genetic variability in coronary artery disease.

References:

1) Jiang H et al. Clin Chim Acta. 2009; 400:3-7;

2) Little J et al. Genet Epidemiol. 2009; 33:581-98.

Grant support: Czech governmental funding ME-856, MSM ME6198959205.

Memory Stick

ABDOMINAL CIRCUMFERENCE AS PREDICTIVE FACTOR OF UNBALANCED METABOLISM AND ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS PATIENTS

Teodora Alexescu[1], V. Negrean[1], N. Rednic[2], F. Eniu[2], A. Cozma[1]

[1]University of Medicine and Pharmacy Iuliu Hatieganu, [2]Clinic CF Hospital, Cluj-Napoca, Romania

Objective: We proposed to investigate if the distribution of the adipose mass may be correlate with the HbA1c and hs - CRP levels in type 2 diabetic patients.

Material and method: The research was performed between may-september 2007 on 30 patients with type 2 diabetes mellitus. Lipid profile (total cholesterol, HDL, LDL, triglycerides), glucose metabolism (a jeun glycemia, A1cHb), anthropometrical parameters (AC, BMI) and hs-CRP level were assesed.

Results: All the patients with increased weight had AC over 80 cm (in females) and over 94 cm (in males). Only 2 of the obese patients had hs - CRP 3 mg%, but 60% of the obese patients and one with overweight had hs - CRP at 1mg%.All of them presented with cardiovascular disease. We found high levels of glycated haemoglobin (8 g%) in all patients with impared glucose tolerance, in over 50% of the patients with second-degree obesity and in 80% of the patinets with third-degree obesity.

Conclusions: Abdominal circumference is a more reliable marker of the adipose mass than BMI and reflects the risk for the cardiovascular complications in diabetic patients. Glycated haemoglobin level is raised in obese patients, the explanation being an inadequate diet and life-style. Although the normal superior hs - CRP level is considerate at 3 mg%, we found that those diabetic patients with hs - CRP at 1mg% also presented with cardiovascular disease.

Memory Stick

NEURO-VASCULAR ALTERATIONS IN CHRONIC HAPATITIS C PATIENTS TREATED WITH INTERFERON: A CASE CONTROL STUDY

Valentina Vedovetto[1], V. Vedovetto[2], A. Rossato[1], F. Veronese[1], A. Zancan[1], F. Bilora[1]

[1]Cardiovascular and Thoracic Science, Clinica Medica II^-Padua University, Padova, [2]Statistical Analysis, Milan University, Milan, Italy

Background: HCV is one of the most important causes of chronic hepatitis.It is still unclear whether HCV chronic hepatitis correlates to atherosclerosis, and if it can cause endothelial and Autonomic Nervous System (ANS) dysfunctions. It´s still unknown the effect of interferon treatment on this alterations.

Aims: To asses the contradictory impact of chronic hepatitis on atherosclerosis pathogenesis, endothelial function, ANS dysfunctions and the role of HCV virus and interferon treatment in natural history of chronic hepatitis C.

Methods: 76 chronic hepatitis C patients and 76 matched controls were evaluated. 51 were treated with interferon and 25 never received therapy; 25 patients recovered and 51 were still ill.Echo-Color-Doppler was used to estimate intima media thickness (IMT) and endothelial function (FMD-flow mediated dilatation); ANS dysfunction was studied with autonomic tests.

Results: Chronic hepatitis C subjects did not show a significant IMT increase; classic risk factors for atherosclerosis seemed to be more important for IMT. Patients presented a significantly alteration of FMD and of ANS tests.Treated subjects, but still ill, had greater dysfunctions.

Conclusions: Chronic hepatitis C does not seem to influenece the onset of pre-atherosclerotic lesions. FMD seemd to be correlated to ANS dysfunctions. Can ANS dysfunctions correlate to atherosclerosis?

Memory Stick

CORONARY ECTASIA: RISK MARKERS & RISK FACTORS

Mahmoud Soliman, H. el Ezawy

Menofyia, Shebin El Kom, Egypt

Introduction: Coronary artery ectasia, many unanswered questions remain regarding its aetiology, clinical sequalae and management.

Aim: To assess risk markers namely HsCRP and risk factors in patients with coronary ectasia and to compare them with those inpatients with atherosclerotic coronary artery disease.

Subjects and methods: The present study included 49 patients with coronary ectasia (group1) & 49 patients with atherosclerotic coronary artery disease (group11).

All patients underwent:

- History taking, clinical examination, ECG, Echocardiography.
- Lab. Investigations: FBs & PPS, S. creatinine anti HCV& HBs antigen.
- Lipidprofile high sensitivity CRP.
- Coronary angiography by judkins´s technique.

Results: There was no significant difference as regards age and sex between both groups (P value >0.05). Group1 have higher levels of Hs CRP (2.94±2.33 vs 1.86+1.27, p< 0.05), higher total cholesterol (215±47.17vs 196.31±43.19) & hypertension was more prevalent than in group 11 (79.6% vs 59.2%, P< 0.05).

When we compare patients with single vessel ectasia with those with single vessel disease, the former have more triglyceride levels (158.33±45.69 vs 111.46±26.53, P< 0.05) and insignificantly higher HsCRP (3.67±3.39VS 2.62±1.42 p>0.05). when we compare patients with two or more vessel ectasia with those with two or more vessel disease, the former were older(58.6±6.3 vs 54.4±8, P< 0.05), more were hypertensive (88.2% vs 61.1% P< 0.05) and have higher levels of HsCRP (2.62±1.62 VS 1.59±1.12 p< 0.001).

Conclusion: Patients with coronary ectasia have higher levels of Hs CRP. Also, hypertension and dyslipidaemia were more prevalent than in patients with atherosclerotic coronary artery disease.

Memory Stick

THE ROLE OF UNKNOWN RISK FACTORS IN THE MIOCARDIAL INFARCTION

A.A. Rafighdoust[1], A.H. Rafighdoust[2], M. Haghmoradi[3], **Shiva Hoorzad**[3]

[1]Cardiology Department of Imam Reza Hospital, Mashhad University of Medical Sciences, [2]Resident of Internal Medicine, Zahedan University of Medical Sciences, [3]Mashhad University of Medical Sciences, Mashhad, Iran

Background: The role of unknown risk factors in Atherosclerosis is becoming increasingly more significant recently. The aim of this study is to underscore the novel risk factors despite the importance of classic factors.

Method and materials: This is a prospective study on 180 myocardial infarction cases, conducted in the cardiology ward and CCU of Imam-Reza hospital (Mashad-IRAN). These risk factors are evaluated: Hyperlipidemia, Hypertension, Diabetes, smoking, activity, stress, hair of external ear canal and ear lobe crease, age and sex. Then patients without any risk factor or with one or two risk factors were distinguished.

Results: The majority of our patients were old men in the age range of 60-69 years. Amongst all patients 42.2% were smokers, 68.3% were type A personality group, 19% were active, 81% were physically inactive, 37.2% had hairy ear canal, 35% had Hypertension, 21.1% were diabetic, 14.4% had Hyperlipidemia and 30% had positive family history of MI. Of great interest was the fact that of the patients whose case was studied many didn´t have any risk factor or in some cases had only one.

Conclusion: our study didn´t show combination of risk factors including hyperlipidemia, hypertension, diabetes and smoking in 20-29 year age group with MI, and 25% of those in 30-89 year age group were risk factor free. it seems logical to detect other unknown risk factors for ischemic heart disease. amongst the new risk factors,inflammation has an important role,other risk factors that must be assessed are Homocystien, serum amyloid,antibodies against Oxidized LDL.

Memory Stick

INFECTION OF AORTA-FEMORALE PROSTHESIS(BY-PASS) WITH PARTICIPATION OF SIGMOID COLON

Apostolo Limuris, N. Fistas

Vascular Surgery, EUROMEDICA-Blue Cross, Thessaloníki, Greece

The infections of the graft prosthesis for the arterial by-pass procedures are very rare. In the international bibliography there are few cases. In four(4) patients with aorto-femoral by-pass three(3) years after the operation procedure presented infection of the retro-peritoneal region with participation of the sigmoid colon.

First of all was done in asportation of the prosthesis aorto-femorales, suture of the sigmoid colon and drainage of the retro-peritoneal region.

St. Auleus was detected in three(3) patients and E. Cloloe in one patient during the micro-biological inspection.

In the second step we performed a vascularization of the lower limb, building a by-pass from the left sousclaviar artery-left common femoral artery in three(3) patients.

In the other patient we performed femoro-femoral by-pass.

One year later vascularization of the lower limb was still in good condition.

Memory Stick

ACUTE PULMONARY THROMBEMBOLISM A.P.TE. EMERGENT TREATMENT WITH RT-PA

Apostolo Limuris, J. Lazaridis, N. Fistas

Dep't of Vascular Surgery, Euromedica-Blue Cross, Kalamaria-Thessaloniki, Greece

In our institution(Private Clinic) we had 20 cases of pulmonary thromboembolism in a time duration of thirty (30) months. We investigated the etiological factors of the A.P.TE. (Post-operation, patients presenting deficiency of C and S proteins, antithrombin III).

In all patients we did a pulmonary angiography (mean time 2 hours) from the beginning of the first signs of A.P.TE and we found segmental pulmonary embolism located in the right and left lung.

Emergent treatment with rt-PA infu sion has started with 10 mg intravenous injection in 4 min and 40 mg in 60 min. After that we repeated the pulmonary angiography in 7 patients and we also repeated the same protocol of rt-PA infusion administration.

All patients continued with low molecular weight heparin for 7 days and with anti-vit K,drugs controlling the I.N.R. and prothrombin time. We had 3 dead patients for right heart insufficiency. The rest of the patients are in very good condition.

We discuss the etiological factors and characteristics of the thrombosis and the way to correct them.

Memory Stick

PROTEIN C DEFICIENCY IN ELDERLY PATIENTS WITH ISCHEMIC HEART DISEASE AND DYSLIPIDEMIA

Soreyya Urazgildeeva[1], L. Eremkina[2], V. Gurevich[1]

[1]Center of Atherosclerosis and Lipid Disorders, Chair of Cardiology of Medical State Academy n.a. I.I.Mechnikov, [2]Laboratory of Biochemestry, Sokolov's Hospital # 122, St. Petersburg, Russia

Rationale: Deficiency of protein C is considered to be a common cause of thromboembolic events (TE) in elderly. Elderly patients having ischemic heart disease (IHD) associated with combined pathology and protein C deficiency are at high risk for the development of TE. These patients require thorough assessment of their lipid metabolism to determine the proper treatment options.

Objective: The aim of the study was to estimate the characteristic features of lipid disorders in elderly patients with IHD and protein C deficiency.

Material and methods: Blood samples from 30 patients over 60 years old with IHD were examined. Disorders in protein C system was determined by the clotting method. Levels of serum total cholesterol (ChS), triglycerides, high-density lipoproteins (HDL) ChS were evaluated by using BIOCON reagents. Low-density lipoproteins (LDL) ChS was calculated according to the M.Friedewald's formula. Values of serum apolipoproteins (apo-A_1 and apo-B) were determined by the nephelometric method.

Results: More than half of the examined patients had protein C deficiency. Hypercholesterolemia was detected in essentially all the patients. Hypoalphacholesterolemia was revealed in 14 patients. In spite of the high level of LDL ChS, only 5 patients had rise in the apo-B values while the low values of apo-A_1 took place twice as often. One third of the patients had the low apo-A_1 levels associated with protein C deficiency.

Conclusion: Assesment of protein C deficiency and apolipoproteins values can be added to routine lipid tests in elderly patients with IHD to reveal those at high risk of TE.

Memory Stick

EFFECT OF DYSGLYCEMIA AND SMOKING ON SHORT TERM MORTALITY IN PATIENTS WITH ACUTE CORONARY SYNDROME

S. Dwivedi, **Amitesh Aggarwal**, R. Kumar

Department of Medicine/ Preventive Cardiology, University College of Medical Sciences, University of Delhi & GTB Hospital, Delhi, India

Objective: To assess the effect of dysglycemia and smoking on short term mortality in patients with acute coronary syndrome.

Methods: Short term mortality data (during first week of acute coronary episode) of 364 patients with acute coronary syndrome was studied from CCU (Jan to Sep 2009) of the UCMS-GTB Hospital, Delhi. These cases were then analyzed as regards to their smoking and dysglycemic status.

Results: Out of 364 CAD patients, 144 (39.5 %) patients were having dysglycaemia. Out of 144 dysglycaemic acute coronary patients 66.6 % were male. Total mortality in dysglycaemic group was 11.1 % compared to 10.1 % in euglycemics. Mortality in dysglycemic males was 10.4 % compared to 8.9 % in euglycemic males. Mortality in dysglycaemic smokers males were 11.1 % compared to 6.6 % in dysglycaemic nonsmokers males and 10.1 % in euglycaemic smokers males. Mortality in dysglycemic females was 12.5 % compared to 14.3 % in euglycemic females. Mortality in dysglycaemic smokers female was 33.3 % compared to 9.3 % in dysglycaemic nonsmokers females and 100 % in euglycaemic smokers females.

Conclusions: Our data corroborate that dysglycaemia accounts for excess mortality compared to euglycemic acute coronary patients. Smoking attributes to higher mortality in dysglycaemic patients in both genders. Short term mortality rates were found to be lower in dysglycemic females compared to euglycemic females. The apparent gender difference in mortality in dysglycemics needs to be looked into in a lager sample over a longer period. Interestingly, both smoking and dysglycemia are modifiable risk factors.

Memory Stick

CORRELATION OF METABOLIC SYNDROME AND CEREBRAL VASCULAR STROKE IN ELDERLY PATIENTS

Charalampos Charalampous, S. Patiakas

General Hospital of Kastoria, Thessaloníki, Greece

Object: To study the possible correlation between Metabolic Syndrome (MS) and Cerebral Vascular Stroke (CVS) in elderly people.

Material - method: 64 patients, with proven ischemic type CVS were the first study group. In these patients MS was assessed by the NCEP-ATP criteria Another 58 patients, aged over 60 years, with proven MS (NCEP-ATP criteria), were the second study group. In this group, this is monitored in the outpatient lipids and hypertension clinic, a recent or ulterior or the existence of CVS was sought in their history.

Results: 38 patients (59.4%), from the first study group with a history of CVS, had MS. Out of the 38, 25 (64.1%) were male and 13 (52%) female. It is worth mentioning that 9 (75%) out if 12 patients who had suffered a stroke in the past, presented MS. From the second study group, with proven MS, a positive history of CVS was established in 3 patients (5.2%), a very high percentage compared to the general population.

Conclusions: It is proven, therefore, that there is a fairly important correlation between MS and CVS in elderly patients. It is crucial to emphasize in the systematic and scholastic information of the public for the dangers of obesity, even from childhood - adolescence, (given the fact that child obesity, who usually leads to adult obesity, is increasing in our country), in order to reduce in the future the prevalence of MS in the general population.

Memory Stick

CHANGE ON PREVALENCE OF METABOLIC SYNDROME RISK FACTORS IN CZECH ARMY DURING LAST 10 YEARS

P. Hlubik, **Hana Stritecka**

Military Hygiene, University of Defence, Hradec Kralove, Czech Republic

Objective: Metabolic syndrome risk level is elevated not only in patients with established CVD, but also with DM II and obesity. The report predicts that unless action is taken, by the year 2020 there will be five million deaths attributable to overweight and obesity, compared to three million now. In Czech Army is program of primary preventive care (PPC) - this is early PPC for soldiers assorted professions, functions and education in age group (25, 30, 33, 36, 39, 40-55 year).

Anthropometrical, laboratory and clinical knowledge give sufficiency information about everyone and so that is real to on this information determine individual medication - preventive recommendation. Sense of this is accepted and long-time kept recommendation and put behind manifestation of civilization diseases (Diabetes mellitus II. type, disorders of metabolisms, hyperlipoprotenemie, hypertension, atherosclerosis….). It is impossible to mention negative influence next risk factors as stress, smoking, overweight, obesity, bad eating habit, live style and aging.

Aim of study: Our objective was the investigation of the change of selected anthropometrical and biochemical parameters, especially those, which are generally, used as risk indices for the origin and development of no infection diseases: BMI, waist circumference, % of body fat, serum concentrations of total cholesterol, HDL and LDL during the period 1999 - 2009.

Supported by VZ MOO FVZ 0000502

Memory Stick

EFFICIENCY OF SECONDARY PREVENTION IN POPULATION WITH CORONARY ARTERY DISEASE IN DNIPROPETROVSK REGION, UKRAINE

Olexandr Kuryata[1], O. Soya[1], N. Bardachenko[2]

[1]Therapy Dept., Dnipropetrovsk State Medical Academy, [2]Cardiology, Dnipropetrovsk State Medical Academy Regional Hospital n.a. Mechnikov, Dnipropetrovsk, Ukraine

Background: Prevention and risk management of atherosclerotic vascular disease remain major health challenges. Coronary artery disease (CAD) and other cardiovascular diseases remain the primary causes of mortality and morbidity in developed countries. The objectives of our study were to determine the prevalence of CAD and myocardial infarction (MI) and adherence for lipid-lowering therapy among Ukrainians in large industrial Dnipropetrovsk region (population more than 3.5 millions).

Methods: We analyzed the coronaroangiography data of 2286 patients with CAD from our region during 2007-2008 years. Primary analysis included age, cholesterol and blood pressure level.

Results: The mean age of patient with CAD was 57.7+/-0.69 yrs, 62.2% had MI in past. 12.4% patients had diabetes mellitus. In 51.2% patients we found uncontrolled high blood pressure and 85.6% had uncontrolled cholesterol level (mean cholesterol level 5.2+/-0.04 mmol/l). We divided the patient after MI depending with age in 4 groups: 4.58% - younger 45 years, 26.38% - 45-59 yrs, 46.54% - 60-74 yrs, 22.5% - 75 yrs and older. According to coronaroangiography data in group younger 45 years in 13.6% patients had stenosis two coronary arteries, in 23.7% - had multivessel disease with stenosis three and more arteries.

Conclusion: We noticed increasing the prevalence of MI with multivessel damages in younger population in combination with uncontrolled cholesterol level. Thus, secondary prevention of coronary atherosclerosis should be improving by optimization statin therapy.

Memory Stick

3 YEAR AGGRESIVE LIPID LOWERING THERAPY OF PATIENTS WITH CHRONIC CORONARY HEART DISEASE

Tamar Vakhtangadze[1], M. Akhvlediani[1], D. Gachechiladze[1], N. Gakhokidze[2]

[1]Clinical Medicine, [2]Medical School Ayety, Tbilisi, Georgia

Objective: Evaluation of impact of agressive lipid lowering treatment on clinical outcomes, CAIM thickening, blood lipids, myocardial SPECT.

Material and methods: 78 consecutive male patients with mild to moderate stress-inducible ischemia on Tc99m Sestamibi Gated SPECT were included in the study. We evaluated the effect of intensive lipid-lowering therapy on coronary symptoms, quality of life, stress-inducible defect size, carotid arteries intima-media (CAIM) thickening and lipid profile. Patients were receiving Atorvastatin 20-80 mg daily maintaining goal of LDLCh in range 65-70 mg/dl with a median follow-up of 36 months. Blood lipid profile and carotid arteries ultrasound, Tc99m sestamibi gated SPECT were performed at the beginning of the study and after every six months. All of the patients were under observation during 36 months.

Results: 1 patient developed stroke during observation. 2 patients underwent revascularisation because of worsening of coronary symptoms. Statin therapy reduced serum LDLCh (-52%; P< 0.001) in all patients; thickening of CAIM and/or plaque size also decreased in 96% of patients by the end of the study. All of the patients showed improvement of symptoms of CAD, which correlated very well with levels of LDL and CAIM. However, gated SPECT showed improvement in stress-inducible defect size in only 37% of patients, whereas there were no changes in 14 patients and other 7 revealed even worsening in reversible defect sizes and perfusion scores.

Conclusion: Statin therapy has good effect on CAD symptoms, outcomes, lipid profile, CAIM, however there is less effect on the perfusion scores acquired by gated SPECT.

Memory Stick

EFFECT OF ATORVASTATIN IN PATIENTS WITH SYSTOLIC HEART FAILURE DUE TO ISCHEMIC CARDIOMYOPATHY AFTER MYOCARDIAL INFARCT

Tamar Vakhtangadze, F. Todua, M. Razmadze, M. Akhvlediani

Clinical Medicine, Tbilisi, Georgia

Purpose: To identify the effect of Atorvastatin + conventional therapy of heart failure on ejection fraction in patients with ischemic dilated cardiomyopathy developed after myocardial infarct.

Methods: 42 consecutive patients with ischemic systolic left ventricle dysfunction (LVEF ≤ 18 - 30%, mean 23%) were enrolled in the study. All of them had previous history of myocardial infarct.

Inclusion criteria: History of MI, fixed perfusion defect in Gated SPECT, low EF detected by Gated SPECT/echocardiography.

Lipids and echocardiography was performed at the beginning of the study, after 6 months of conventional therapy and by the end of the study. All patients received conventional heart failure treatment for 6 months before initiation of Atorvastatin treatment. Mean time of follow-up was 27 months (12- 36 months). Atorvastatin was given to all of them after 6 months of enrolment in the study.

Results: Patients developed improvement of symptoms of HF after 6 months of conventional heart failure treatment - mean EF has risen from 23 to 27%. Atorvastatin addition to conventional treatment led to additional improvement of EF in 72 % of patients, which was more clear after 1 year of treatment - mean EF has risen from 23% to 36% by the end of the study. Patient who did not respond to Atorvastatin treatment had larger perfusion defects and more severe coronary arteries disease than patients who respond to Atorvastatin treatment.

Conclusion: Atorvastatin addition to conventional heart failure treatment leads to improvement of EF in patients with heart failure due to ischemic cardiomyopathy.

Memory Stick

ADVENTITIAL CALCIFICATIONS:SURPRICE LOCATION. ATHEROSCLEROTIC AND CALCIFIED PLAQUES BEGIN AND GROW UP NOT ONLY BENEATHTHE ENDOTHELIUM, BUT ALSO ON THE ADVENTITIA

Faruk Erzengin

Cardiology, University of Istanbul, Istanbul Medical Faculty, Istanbul, Turkey

Multislice computed tomography (MSCT) is an important tool for the non-invasive evaluation. We have recently shown that by MSCT the formation of ateromatous component, atherosclerotic or calcified plaques start and grow up not only under the endothelium, but also just beneath the Adventitia. Most patients (55.1%), the calcified plaques begin just beneath of the epithelium on the Adventitia and grow up to ward of the lumen. MSCT is useful for the characterization of human coronary plaque morphology by determining tissue density within the lesion non-invasively. Also MSCT is a unique method for the diagnosis of dangerous silent myocardial ischemia by lumen narrowing calcified plaques. Interestingly, the formation of calcified plaque much frequently begins beneath the sub epithelium of the adventitia and quickly grows up towards to the lumen and keeping classical atherosclerotic cascade on the coronary arteries. The cholesterol (oxLDL) and macrophage easily arrive to the Adventitia by vase vasorums. Molecular imaging most probably will be sort it out this morphology in the near feature. In this study, randomised 98 cases (38 F, 60 M) were investigated making a comparison between MSCT (using with computerised magnifying glass) and conventional coronary angiography. Stents implantations were performed 66 of cases, 22 of patients underwent CABS and 10 patients were treated medically. In this study we have described that Adventitial location of atherosclerotic, fibrotic or calcified plaques' formation begin not only from endothelium but also mostly (55.1%) from the Adventitia. This finding is firstly described in the Literature.

Memory Stick

CORONARY ARTERY CALCIUM IS AN IMPORTANT RISK INDEX FOR ARTERIAL HYPERTENSION PATIENTS

Tatjana Kuznetsova[1], D. Gavrilov[2], S. Madany[3], I. Dudanov[4]

[1]Faculty Therapy, Petrozavodsk State University, Petrozavodsk, [2]Cardiology, [3]Therapy, Medical Center, Kondopoga, [4]Surgery, Petrozavodsk State University, Petrozavodsk, Russia

273 arterial hypertension patients (AH) have been examined (average age of 53,3±6,9, 29% - women). Standard examination was conducted. Additional risk factors (RF) were evaluated: smoking - 136 patients, AH family history - 194, obesity - 111, dislypidaemia - 142, diabetes mellitus - 23 patients, combination of more than 3 RF - 164. Left ventricular hypertrophy was found in 37%, microalbuminuria - 2,3%, ultrasound signs of carotid artery involvement - 79% (plaques in 49%). 51 patients had history of ischemic heart disease (IHD), 17 - stroke, 7 - peripheral atherosclerosis. Coronary artery calcium (CAC) was calculated by 64-row multidetector computer tomography. CAC was found in 54%, calcium index was higher than the age norm in 38%.

CAC was associated with sex (OR 2,8, p=0,0001 in men), age after 50 (OR 2,1. p=0,003). CAC wasn't connected with smoking (OR 1,5, p=0,08), family history (OR 1,2, p=0,4), obesity (OR 1,5, p=0,1), dislypidaemia (OR 0,8, p=0,3), diabetes (OR 1,1, p=0,8). CAC was associated with the combination of more than 2 RF (OR 2,6, p=0,01). CAC correlated with ultrasound signs of atherosclerosis (OR 2,3, p=0,006), IHD (OR 2,6, p=0,0036). Risk assessment by CAC correlated with the SCORE scale (OR 2,5, p=0,0002).

CAC in AH patients is a reliable method of risk assessment, comparable with SCORE, mostly after 50 years old, in men, with the combination of 2 and more RF. Ultrasound signs of carotid lesion are the marker of coronary calcium.

Memory Stick

INFLUENCE OF ENHANCED EXTERNAL COUNTERPULSATION ON ANGIOGENESIS INDUCED FACTORS

Igor Sergienko[1], S. Gabrusenko[2], V. Malakhov[2], V. Masenko[3], V. Kukharchuk[2]

[1]Atherosclerosis, Russian Cardiology Research and Production Center Cardiology Research Complex, [2]Atherosclerosis, [3]Immunology, Russian Cardiology Research and Production Center, Moscow, Russia

Aim: At present Enhanced external counterpulsation (EECP) is used in clinical practice for treatment patients with refractory angina pectoris, but the mechanism remains unclear. The aim of our investigation was to assess the dynamic of vascular endothelial growth factor (VEGF), transforming growth factor β (TGF β), brain and atrium natriuretic peptide (BNP and ANP) after EECP treatment.

Material and methods: 42 patients (38 mail, 4 female, mean age - 62,9±8,1) with ischemic heart disease -stable angina pectoris - were treated by EECP on. The treatment duration was 35 h for each patient. Before and after treatment we measured the serum level of VEGF, TGF β, BNP and ANP. The myocardial perfusion was assisted by single photon emission computer tomography (SPECT).

Results: There was significant increase of exercise test duration till stress-induced myocardial ischemia onset, reduce of chest pain episodes and decreasing of nitrates consumption. SPECT demonstrated decreasing of perfusion defects severity from 64,86±14,01 std till 58,5±19,9 std, p< 0,01.

The VEGF level significantly increased after EECP treatment - 287,30 (219,70-417,25) pg/ml before and 353,50 (281,90-512,10) pg/ml after, p< 0,05. But TGF β level didn't change - 4,51 (3,34-5,48) and 4,20 (3,02-6,18) ng/ml accordingly.

Initial level of ANP was 570,33 (412,39-650,45 pg/ml, after treatment it decreased - 354,12 (177,37-615,88) pg/ml, p< 0,05. BNP β level didn't change - 526,84 (255,58-657,20) and 471,12 (253,88-579,16) ng/ml, p>0,05.

Conclusion: This study shows that EECP treatment influences on exercise duration and myocardial perfusion. These improvements can be determined by coronary angiogenesis mechanisms stimulation.

Memory Stick

IDENTIFICATION OF NOVEL PROTEIN BIOMARKERS FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

Seraina Decasper[1], D. Hof[1], B. Biedermann[2], A. von Eckardstein[1]

[1]Institute of Clinical Chemistry, University Hospital Zurich, Zurich, [2]Molecular Nephrology, Department of Research, University Hospital Basel, Basel, Switzerland

The early diagnosis of acute coronary events suffers from low diagnostic sensitivity of myocardial necrosis markers and the identification of patients at high cardiovascular risk is limited by the low predictive positive value of risk factors. At least in theory both the diagnostic and prognostic efficacy may be improved by biomarkers that are released from the atherosclerotic lesion into the blood for example by plaque ruptures or erosions.

Subtractive antibody phage display was used to identify proteins that are specifically present in advanced atherosclerotic lesions. As we want to find a biomarker present in blood we are working with so-called secretomes, i.e. the array of proteins secreted from arterial tissues which are classified according to the severity of the lesion and incubated in protein-free medium for 24 hours. During this time, proteins were secreted or diffused out into the medium, which was used as an antigen mixture for antibody phage display screening.

In our selections we found several phages that specifically recognize proteins in the secretome from atherosclerotic plaque tissues in ELISA and western blot analysis.

We have now recloned the antibodies to express them as soluble recombinant antibodies. Now we will use the antibodies for characterizing and identifying their antigens by immunohistochemistry on tissue slides, western blotting of secretomes and plasma as well as mass-spectrometry.

Memory Stick

DETERMINANTS OF CORONARY PLAQUE COMPOSITION BY COMPUTERIZED CORONARY TOMOGRAPHY ANGIOGRAPHY IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

Marcio Hiroshi Miname, M.S. Ribeiro, F. Ávila, J. Parga, C.E. Rochitte, R.D. Santos

Heart Institute (InCor) - University of São Paulo, São Paulo, Brazil

Introduction: Previous studies showed a high prevalence of subclinical atherosclerosis in heterozygous familial hypercholesterolemia (FH).

Objective and methods: Our objective was to evaluate the association of coronary plaque composition with clinical and laboratorial parameters, arterial stiffness (pulse wave velocity-PWV and carotid distensibility-CD), and with carotid intima-media thickness (IMT) in asymptomatic FH subjects using 64-slice CT coronary angiography.

Results: we studied 102 FH subjects (45±13 years-old, 36% men, LDL-c 280±54mg/dL). Forty eight presented coronary plaques, 26% had non-calcified plaques, 29% had mixed plaques and 35% had calcified plaques. The prevalence of segments with mixed (p=0.022) and calcified plaques (p=0.0003) increased with age, while segments with non-calcified plaques not (p=0.29). In univariate analysis, the following variables were related to non-calcified plaque presence: male gender (48% vs. 9%, p=0.0045), family history of premature coronary heart disease (CHD) (70% vs. 41%, p=0.038) and higher hip circumference (96±8 vs. 101±11cm, p=0.049). Multivariate analysis showed that only male gender was related to non-calcified plaque (OR:15.45; CI95%: 1.72-138.23, p= 0.014), only premature family history of CHD was related to mixed plaque presence (OR=4.9; CI95%: 1.32-18.21, p=0.018) and male gender was related (negatively) to calcified plaque presence (OR=0.21; CI95%: 0.05-0.84; p=0.027). IMT, PWV, CD and lipid profile were not associated with coronary plaque composition.

Conclusion: Male gender and family history of premature CHD were associated with non-calcified and mixed plaques, respectively, in FH subjects. Men have less chance of calcified plaque presence than women.

Memory Stick

COMBINATION OF NIGELLA SATIVA AND LOW DOSE REPAGLINADE RETARDS THE PROGRSSION OF ATHEROSCLEROSIS

Bassim Mohammad[1], N. Hadi[2]

[1]Pharmacology and Therapeutics, College of Medicine, Al Qadisiyah University, Diwaniyah, [2]Pharmacology and Therapeutics, College of Medicine, Kufa University, Kufa, Iraq

Background: Atherosclerosis is responsible or morbidity and mortality worldwide.

Aim: This study was undertaken to clarify the effects of nigella sativa and low dose repaglide on progression of atherosclerosis.

Materials and methods: A total of 40 New Zealand White Male Rabbits were used in this study. These animals were randomized into 5 groups, 8 rabbits each. The animals in group 1 were maintained on standard chow diet (normal diet control) while those in group 2 received atherogenic diet (atherogenic control) throughout the experiment (12 weeks). In addition to atherogenic diet, rabbits in group 3, 4 and 5 were treated orally with n-hexane extract of nigella sativa (N) (10 g/kg/day), repaglinade (R) (0.3 mg/kg/day) or their combination (N+R) respectively. The blood samples were taken to measure lipid profile, haemostatic parameters (PT, platelets count and plasma fibrinogen level), oxidation parameters (serum MDA level, GSH level and SOD activity) and SUA (serum uric acid). In addition, histomorphometery were done to asses the aortic intimal thickness.

Results: Plasma fibrinogen was significantly reduced by N, R and N+R ($p< 0.05$, $p< 0.05$ and $p< 0.01$ respectively). Significant ($p< 0.05$) prolongation in PT was only observed in N+R group. N, R and N+R significantly reduced serum MDA level ($p< 0.05$, $p< 0.05$ and $p< 0.001$ respectively) and decreased serum SOD activity ($p< 0.05$). N+R significantly increased serum GSH level ($p< 0.001$). Aortic intimal thickness was significantly reduced by treatment with N ($p< 0.05$) and N+R ($p< 0.01$).

Conclusions: Nigella sativa potetiated antiatherosclerotic effect of repaglinade.

Memory Stick

HS-CRP AND ANTI-PHOSPHORYLCHOLINE IGM ANTIBODIES: BIOMARKERS FOR RISK ASSESSMENT OF CVD

Vania Polymerou[1], S. Kanakari[2], L. Fountas[2], D. Lavranos[2], J. Kremastinou[3]

[1]Biopathology, [2]Biomed Diagnostic Laboratories, [3]National School of Public Health, Athens, Greece

Background: Atherosclerosis is an inflammatory process in the artery wall and the main cause of Cardiovascular Disease. LDL and hs-CRP levels are generally accepted as significant biomarkers for CVD. Recent clinical data support that IgM antibodies against phosphorylcholine (anti-PC IgM) at low levels could also be used as a novel biomarker for CVD. The aim of this study is to evaluate the correlation between hs-CRP, LDL and anti-PC IgM.

Materials and methods: The sample of our study consists of 95 patients with increased LDL levels (>170 mg/dL). All samples were tested for hsCRP and anti-PC IgM serum levels. Elecsys 2010 and Nephelometry were used to measure LDL concentration and hs-CRP levels, respectively. An ELISA kit (CVDefine, Athera AB) was used to determine anti-PC IgM levels. Samples were also tested for HBA1C and Glucose levels using HPLC and Electrochemiluminescence respectively. In the statistical analysis the nonparametric Mann-Whitney's test was used. Differences at $p< 0.05$ were considered as statistically significant.

Results: Hs-CRP levels showed a significant positive correlation to LDL (R=0,846, p=0,001), while limited correlation was found between anti-PC IgM levels and LDL (R=0,435, p=0,05). The presence of diabetes mellitus did not show any association with anti-PC IgM and hs-CRP. Positive correlation was found only between LDL and Glucose serum levels (R=0,531, p=0,005).

Conclusion: This study suggests that hs-CRP in addition to lipid profile are very useful to evaluate the risk for CVD. Anti-PC IgM levels may help in the evaluation of CVD, but further studies are needed to confirm its role and importance.

Memory Stick

STUDIES ON HDL-ASSOCIATED ENZYMES AND SCAVENGER RECEPTORS UNDER EXPERIMENTAL HYPERCHOLESTEROLEMIA: POSSIBLE MODULATION ON SELENIUM SUPPLEMENTATION

Harmandeep Kaur, M.P. Bansal

Department of Biophysics, Panjab University, Chandigarh, India

Atherosclerosis is a chronic disorder of the arterial walls. High-density lipoproteins (HDL) blood levels are inversely correlated with atherosclerosis and this beneficial effect of HDL has been partly attributed to its antioxidant properties mediated by paraoxonase1 (PON1) or platelet-activating factor acetylhydrolase (PAF-AH). In addition, scavenger receptors (SR) play an important role in atherogenesis. Thus, present study was aimed to investigate the role of the trace element selenium (Se) on the expression of HDL-related antioxidant enzymes and SRs in experimental hypercholesterolemia. Male Sprague Dawley rats were fed standard control, high fat diet (HFD) with/without Se supplementation (1ppm) for 4 months after which various parameters were analyzed in serum and liver. Cholesterol, triglycerides, HDL and LDL levels were significantly increased by HFD, whereas serum Se levels in HFD group were reduced compared with control group. Se levels recovered in rats fed a HFD + Se and Se supplementation lowered the triglycerides level. ROS in the liver were 2-fold increased by HFD and Se supplementation however, diminished the levels. Furthermore, Se also improved the HFD-mediated reduction of serum PON1 enzyme activity and PON1 protein levels. HFD significantly increased hepatic CD36 mRNA and protein expression as well. This transcriptional up-regulation of CD36 was inhibited in rats receiving Se in addition to HFD. Thus, Se supplementation might be a valuable approach as it appears to be protective in hypercholesterolemia by restoring the antioxidant properties of the HDL associated enzyme PON1 and by blocking the up-regulation of the pro-atherogenic SR CD36 in the liver.

Memory Stick

EXTENDED RELEASE NIACIN/LAROPIPRANT IS PROJECTED TO REDUCE CORONARY RISK BEYOND STATINS: COST-EFFECTIVENESS ANALYSIS IN SECONDARY PREVENTION PATIENTS IN GERMANY

Galin V. Michailov[1], G.M. Davies[2], B.M. Ambegaonkar[3], K.J. Krobot[1]

[1]Outcomes Research, MSD Sharp & Dohme GmbH, Haar, Germany, [2]Health Economic Statistics / Merck Research Laboratories, Merck Sharp & Dohme Corp., Upper Gwynedd, PA, [3]Global Health Outcomes, Merck Sharp & Dohme Corp., Whitehouse Station, NJ, USA

Background: Despite statins significant risk for coronary events remains. We aimed to project added years of life and the cost-effectiveness of extended release niacin/laropiprant (ERN/L) 2g/d for coronary heart disease (CHD) patients not at LDL-cholesterol goal on simvastatin alone.

Methods: CHD patient profiles (age 50-80 years) with LDL-cholesterol ≥ 100 mg/dL on simvastatin in 2007 were selected from a population-based primary care database in Germany (IMS Health). Lifetime benefits were projected using the Framingham algorithm and federal non-CHD mortality statistics for 2007. Subgroup analyses included CHD patients with and without diabetes mellitus, above and below age 65 years, outside the normal HDL-levels defined by the European Society of Cardiology (HDL-cholesterol < 40 mg/dL in men; HDL-cholesterol < 45 mg/dL in women) or with total cholesterol/HDL-cholesterol ratio ≥ 4.5.

Results: A total of 886 patients on simvastatin 20 mg and of 707 patients on simvastatin 40 mg were included (mean age 68 years; 39% female). ERN/L added to simvastatin 40 mg was estimated to gain 0.80 life-years compared to simvastatin 40 mg alone. ERN/L added to simvastatin 20 mg was estimated to gain 0.53 life-years compared to doubling simvastatin dose from 20 mg to 40 mg. ERN/L was projected to be cost-effective with estimated cost-effectiveness ratios below 27,000 €/LYG in all scenarios including sensitivity analyses.

Conclusion: Based on these projections ERN/L is estimated to add substantial life years in CHD patients not at LDL-cholesterol goal on simvastatin and appears to be a cost-effective option for these patients across all subgroups.

Memory Stick

INHIBITION OF ENDOTHELIAL AND MONOCYTIC CELL MIGRATION BY RESVERATROL: IMPLICATIONS FOR ATHEROSCLEROSIS

Iwona Cicha, M. Regler, K. Urschel, W.G. Daniel, C.D. Garlichs

Department of Cardiology and Angiology, University of Erlangen-Nuremberg, Erlangen, Germany

Objectives: The anti-inflammatory red wine polyphenol, resveratrol, was implicated in cardiovascular protection. We have previously demonstrated that resveratrol inhibits TNF-alpha-induced endothelial activation and monocyte recruitment. In this study, we investigated the effects of resveratrol on endothelial and monocytic cell migration.

Methods: Human umbilical vein endothelial cell (EC) migration was assessed in a modified barrier assay. EC shape and actin cytoskeleton were analyzed. Chemotaxis of THP-1 monocytes was determined using Boyden chamber, with monocyte chemotactic protein (MCP)-1 as the stimulus. MCP-1 receptor (CCR2) expression was determined by Western blotting.

Results: Treatment with resveratrol (1-20 µmol/L) dose-dependently inhibited the unstimulated EC migration. In the treated cells, a progressive shape elongation was observed, evident after 6h of treatment. These effects of resveratrol were independent of nuclear factor-kappaB, but were abrogated in presence of RhoA kinase inhibitors, indicating a novel mechanism of resveratrol activity in EC.

In THP-1 monocytes, treatment with resveratrol dose-dependently inhibited the chemotaxis towards MCP-1, with significant effect already at 5 µmol/L. At resveratrol concentration of 50 µmol/L, chemotaxis was reduced nearly to negative control levels. However, only a slight reduction in protein expression of CCR2 was observed by Western blot analysis in resveratrol-treated cells. This indicates that resveratrol prevents monocyte chemotaxis via inhibition of receptor activation or the inhibition of signalling downstream of CCR2.

Conclusions: Resveratrol dose-dependently inhibited unstimulated endothelial cell migration and MCP-1-induced monocytic cell chemotaxis. This activity may contribute to the cardioprotective effects of resveratrol by inhibition of intimal neovascularization and monocyte recruitment into the artery wall.

Memory Stick

LXR ACTIVATION WITH TO901317 AND GW3965 IN MICE: DIFFERENTIAL EFFECTS ON LIVER AND INTESTINE, BUT NO NEGATIVE IMPACT ON BONE

Janne Prawitt[1], F.T. Beil[2], B. Staels[1], J. Heeren[3], A. Niemeier[3,4]

[1]Departement d'Atherosclerose, Institut Pasteur de Lille, Inserm U545, Univ Lille Nord de France, UDSL, Lille, France, [2]Department of Osteology and Biomechanics, [3]Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, [4]Department of Orthopaedics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Objective: The nuclear receptor LXR is a regulator of cholesterol homeostasis and its ligands have been suggested for the treatment of atherosclerosis. LXR-deficient mice have been shown to display an increased bone mass, suggesting that LXR activation may have a negative effect on bone. We therefore examined the impact of long term LXR activation on bone in growing mice.

Methods and results: Short-term administration of the LXR agonist TO901317 inhibited mRNA and protein expression of osteocalcin, an osteoblast-specific marker of bone formation *in vitro* and *in vivo*. To assess the long-term effect of LXR activation on bone, the two LXR agonists TO901317 or GW3965 (10mpk respectively) were administered to C57Bl/6 mice for 12 weeks, starting at 4 weeks of age. LXR target genes were differentially regulated in liver and intestine by the two agonists. While TO901317 induced Abca1, Abcg1, Abcg5 and Abcg8 in liver (except for Abca1) and intestine as expected, GW3965 lead to a significant reduction of gene expression in liver and only a weak increase in the intestine. LXR activation did not have a negative influence on bone growth, formation and turnover as measured by total body contact radiography, lumbar trabecular bone histomorphometry, micro-CT of femur cortical bone and biochemical markers of bone turnover.

Conclusion: Long term LXR activation in mice is safe with respect to side effects on bone. The unexpected differential effects of the available synthetic LXR ligands on liver and intestine should be taken into account when considering these drugs as therapeutic agents.

Memory Stick

CLUSTERING BY PLASMA LIPOPROTEIN PROFILE REVEALS LARGE FENOFIBRATE RESPONDER SUBGROUP

Daniël B. van Schalkwijk[1,2], K. van Bochove[1], L.D. Parnell[3], C.-Q. Lai[3], J.M. Ordovás[3], A.A. de Graaf[1], B. van Ommen[1], D.K. Arnett[4]

[1]BU Biosciences, TNO Quality of Life, Zeist, [2]Analytical BioSciences, LACDR/Leiden University, Leiden, The Netherlands, [3]The Nutrition and Genomics Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, [4]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA

Introduction: Fibrates lower triglycerides and raise HDL cholesterol in dyslipidemic patients, but the response to treatment differs between patients. Earlier studies have used baseline triglyceride or HDL cholesterol differences to identify response subgroups.

Objective: We investigated whether clustering by the complete baseline lipoprotein profile identifies a different subgroup with favorable response to fenofibrate treatment than cutoffs used in earlier studies, in subjects from the GOLDN study.

Methods and results: We divided the baseline NMR lipoprotein profiles of 775 participants in the GOLDN study into three subgroups, using k-means clustering. At baseline, the subjects in each subgroup showed differences in presence/absence of cardiovascular risk factors. The clustering found an additional 33% of the population with favorable response to fenofibrate in comparison with baseline triglyceride cutoff subgroups. For HDL cholesterol cutoff, this figure was 18%. Compared to triglyceride subgrouping, the additional responder subjects showed a lower triglyceride response to fenofibrate, but a similar response of HDL and LDL parameters. These responder subjects showed a similar triglyceride and HDL response, as well as a favorable LDL response compared to the HDL cholesterol subgrouping.

Conclusions: We have identified a new subgroup of responders to fenofibrate therapy using lipoprotein profile clustering. The new subgroup considerably enlarges the responder subgroups previously identified with baseline HDL cholesterol and triglyceride cutoffs. These results can help to increase the number of patients that will benefit from fibrate treatment.

Memory Stick

EFFECTS OF INTENSIVE LIPID LOWERING WITH ATORVASTATIN ON PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH CORONARY HEART DISEASE

Mogens Lytken Larsen[1], I. Holme[2], M. Tikkanen[3], R. Fayyad[4], R. Laskey[4], on behalf of the Incremental Decrease in End Points through Aggressive Lipid Lowering (IDEAL) Study Group

[1]Department of Medicine-Cardiology, Arhus University Hospital, Arhus, Denmark, [2]Oslo University Hospital, Ulleval, Oslo, Norway, [3]Helsinki University Hospital, Helsinki, Finland, [4]Pfizer Inc, New York, NY, USA

Background: The benefit of statins in peripheral arterial disease (PAD) patients with CHD is less evident, than in other high-risk patients.

Methods: In the IDEAL trial treatment with atorvastatin 80mg vs simvastatin 20-40mg reduced the relative risk of major coronary events (MCEs) in patients with CHD (n=8888) by 11% (P=0.07). This post hoc analysis of IDEAL investigated the impact of having PAD at baseline, and the effect of intensive lipid-lowering (atorvastatin 80mg) vs simvastatin 20-40mg on the incidence of PAD.

Results: PAD occurred in 127 patients (2.9%) receiving atorvastatin and 167 patients (3.8%) receiving simvastatin, representing a 24% reduction in relative risk (HR=0.76,95%CI 0.61-0.96;P=0.02). At baseline, 4.2% of patients had a history of PAD. The risk of MCEs was ~2-fold higher in patients with PAD at baseline vs those without PAD (HR=1.92, 95%CI 1.49, 2.47;P< 0.0001). Among patients with PAD at baseline, the rate of MCEs during the study was lower in those receiving atorvastatin (14.4%) vs simvastatin (20.1%) (HR=0.68,95%CI 0.41-1.11;P=0.125). In this patient group, a significant treatment effect with atorvastatin was seen for reduction of any coronary event (P=0.04), revascularization (P=0.007), and any cardiovascular event (P< 0.05), but not for recurrent PAD (p=0.605).

Conclusion: In IDEAL, intensive treatment with atorvastatin significantly reduced the incidence of PAD compared to standard therapy with simvastatin. Patients with a history of PAD at baseline had a significantly higher risk of future cardiovascular events; intensive treatment with atorvastatin significantly reduced the risk of any coronary or cardiovascular event, but not that of recurrent PAD.

Memory Stick

SWITCHING FROM ANY STATIN BRAND TO EZETIMIBE/SIMVASTATIN 10/20 MG IS MORE EFFECTIVE AT LOWERING PLASMA LIPIDS VERSUS ROSUVASTATIN 10 MG

Philippe Brudi[1], M. Averna[2], M. Farnier[3], L. Missault[4], H. Vaverkova[5], L.M. Viigimaa[6], Q. Dong[7], A. Shah[7], W. Taggart[7], A. Johnson-Levonas[7]

[1]Merck Schering-Plough, Whitehouse Station, NJ, USA, [2]University of Palermo, Palermo, Italy, [3]Point Medical Rond Point de la Nation, Dijon, France, [4]St Jan Hospital, Brugge, Belgium, [5]Medical Faculty and University Hospital Olomouc, Olomouc, Czech Republic, [6]Tallinn University of Technology North-Estonia Regional Hospital, Tallinn, Estonia, [7]Merck Research Laboratories, Rahway, NJ, USA

This post-hoc analysis compared the effects of switching from statin monotherapy to ezetimibe/simvastatin 10/20 mg (EZE/SIMVA) vs rosuvastatin 10 mg (ROSUVA) in patient subgroups defined by the brand of pre-randomization statin therapy. In this study, high-risk patients with elevated LDL-C (≥2.59 and ≤4.92 mmol/L) despite statins entered an open-label period and received the same statin brand/dose prior to randomization. Patients were randomized 1:1 to EZE/SIMVA 10/20 mg or ROSUVA 10 mg for 6 weeks. Patient subgroups were defined by the brand of pre-randomization statin therapy (atorvastatin n=232, rosuvastatin n=94, simvastatin n=186 and other [fluvastatin, lovastatin, pravastatin] n=106). This analysis evaluated % change in lipids/lipoproteins in the patient subgroups. Consistency of the treatment effect across subgroups was evaluated by testing for treatment×subgroup interaction without multiplicity adjustment. Treatment effects across the subgroups were generally consistent with the overall population. EZE/SIMVA was more effective than ROSUVA at lowering LDL-C, TC, non-HDL-C and apo B in the overall population and across the subgroups. Irrespective of statin brand, switching from statins to EZE/SIMVA 10/20 mg vs ROSUVA 10 mg provided superior lipid reductions.

Between-treatment differences (EZE/SIMVA-ROSUVA; 95% CI)in LS mean % change from baseline at study endpoint a						
	Overall population	Atorvastatin	Rosuvastatin	Simvastatin	Other	P-value for treatment x subgroup interaction
LDL-C (mmol/L)	-10.7 (-14.1, -7.3)*	-14.3 (-21.2, -7.4)	-11.3 (-23.0, 0.5)	-7.9 (-14.0, -1.8)	-8.0 (-16.0, 0.1)	0.298
TC (mmol/L)	-7.2 (-9.6, -4.8)*	-9.2 (14.0, -4.4)	07.8 (-15.7, 0.0)	-4.8 (-9.2, -0.4)	-4.6 (-10.3, 1.1)	0.438
non-HDL-C (mmol/L)	-9.4 (-12.5, -6.3)*	-10.7 (-16.8, -4.5)	-9.8 (-20.4, 0.7)	-7.7 (-13.7, -1.7)	-8.0 (-15.3, -0.8)	0.723
apo B (g/L)	-8.1 (-10.9, -5.3)*	-9.8 (-15.5, -4.1)	-9.8 (-19.1, -0.6)	-9.4 (-14.7, -4.1)	-4.6 (-10.8, 1.7)	0.852
a Last available post-baseline value during the 6-wk active treatment period; *P<0.001						

[Difference in LS mean % change from baseline]

Memory Stick

ROSUVASTATIN DELAYS PROGRESION OF AORTIC VALVE STENOSIS CAUSED BY HYPERTENSION

Gustavo Giunta[1], E. Guevara[1], L. Marziali[1], G. Yannarelli[1], L. Gomez Rosso[2], T. Meroño[2], R. Favaloro[1], F. Brites[2], L. Cuniberti[1]

[1]Favaloro Foundation - Favaloro University, [2]Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Epidemiological data support that risk factors leading to aortic valve stenosis (AS) are similar to those of vascular atherosclerosis. Recently, mild AS was developed in a normocholesterolemic rabbit model of hypertension. We hypothesized that rosuvastatin (Rosu) treatment modifies this transformation.

Methods: Male NZ rabbits (n=43) instrumented with one-kidney/one-clip hypertensive model were randomized to: HT (n=17) with regular diet; HT+R (n=14) with Rosu (2mg/kg/d) + regular diet; and HT+R+C (n=12) with Rosu (2mg/kg/d) + individual cholesterol supplementation in diet to maintain basal blood cholesterol levels. A control (CT) group (n=15) received sham surgery. Systolic (SBP) and diastolic blood pressures (DBP) were measured by direct method. Aortic valve (AV) characteristics were assessed by echocardiography, at baseline, 3 and 6 months of hypertension.

Results: After 6 months of follow-up, SBP and DBP increments from baseline were highest in HT (49% and 40%; respectively, $p< 0.001$), and much lower in Rosu groups (SBP=23 and 25%; DBP=28 and 26%; $p< 0.001$ for HT+R and HT+R+C, respectively). Total cholesterol decreased 45.7% ($p< 0.01$) only in HT+R. The AV thickness was increased in HT (0.5±0.09 vs. 0.6±0.15mm; $p< 0.01$), without difference in HT+R and HT+R+C. Finally, AV area showed a significant decrease in HT (0.277±0.01 vs. 0.208±0.06 cm^2, $p< 0.05$), no difference in HT+R and HT+R+C; and a non-significant increase in CT (0.264±0.08 vs. 0.32±0.06 cm^2, $p=0.07$).

Conclusions: This preclinical study supports a potential benefit of Rosu treatment in the development of AS induced by hypertension. Moreover, this effect could be attributed to a non-lipid lowering mechanism.

Memory Stick

ARTERIAL BAROREFLEX FUNCTION IS A NEW TARGET OF ATHEROSCLEROSIS

Guo-Jun Cai

College of Pharmacy, Second Military Medical University, Shanghai, China

The present study was designed to testify the hypothesis that arterial baroreflex function is a new target of atherosclerosis in spontaneously hypertensive rats. Experiment 1: the baroreflex sensitivity (BRS) was measured in 31 spontaneously hypertensive rats (SHR) in conscious state with a computerized blood pressure monitoring system. Four weeks later, the rats were administered with Vitamin D_3, and fed with the high-cholesterol diet for 8 weeks to induce atherosclerosis. Then their hearts and aortae were removed for pathological examination. A negative correlation was found between BRS and the scores of coronary ($r=-0.460$, $P< 0.01$) or aortic atherosclerosis ($r=-0.448$, $P< 0.05$) in SHR. Experiment 2: SHR were administered with Vitamin D_3 and fed with the high-cholesterol diet; simultaneously half of them were treated with low-dose ketanserin. The atherosclerosis scores of treatment group were significantly lower than control group (coronary score: 0.90 ± 0.14 versus 1.76 ± 0.27, $P< 0.05$; aortic scores: 1.00 ± 0.39 versus 2.18 ± 0.41, $P< 0.05$). Experiment 3: Male New Zealand White rabbits were fed with the high-cholesterol diet; and some of them were treated with low-dose ketanserin at the same time. The atherosclerosis scores of treatment group were significantly lower than control group (aortic scores: 0.26 ± 0.08 versus 0.60 ± 0.11, $P< 0.05$). These results indicated that arterial baroreflex function may be a new target of atherosclerosis.

Keywords: Atherosclerosis; Baroreflex; SHR; ketanserin.

Memory Stick

ROLE OF CARVEDILOL WITH TOCOTRIENOL TO EXTENUATE ANTHRACYCLINE INDUCED CARDIOMYOPATHY IN RATS

Mallika Jainu[1], V. Mohan[2]

[1]Department of Biomedical Engineering, SSN College of Engineering, (affiliated: Anna University), [2]Department of Biochemistry, University of Madras, Chennai, India

Doxorubicin (DOX), an anthracycline antibiotic, has been widely used in the treatment of a variety of tumors and carcinomas; however its clinical application is limited by its irreversible cardiac side effect of congestive heart failure. This study is directed to investigate the possible synergistic effect of carvedilol, a non-selective α_1 and β-adrenergic antagonist and tocotrienol against doxorubicin induced cardiomyopathy in rats. Interestingly, carvedilol and tocotreniol was accompanied by a significant negative correlation with cardiac hypertrophy and leak of cardiac troponin T into serum. In the present study, elevated TBARS were associated with reduced tissue catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activities in the aorta of doxorubicin administered rats. Aorta GSH, CAT and SOD enzymes was increased and simultaneous reduction in the levels of TBARS was noted in carvedilol and tocotreniol treated rats. The combination therapy increased Bcl-2/Bax expression ratio and decreased caspase-3 activity in cardiomyopathy induced rats, which resulted in reduction of apoptosis as evidenced by western blotting. Carvedilol and tocotreniol treatment reversed these levels, prevented the hypertrophic cardiac histology and restored the normal ultrastructural architecture. This study document the mechanisms behind the beneficial efficacy of carvedilol and tocotrienol on cardiotoxicity, which can throw a new light on cancer therapy with an associated reduction in apoptotic cell death *in vivo*.

Memory Stick

DIABETIC CARDIOMYOPATHY IN TYPE 2 DIABETIC PATIENTS: EFFECTS OF ADMINISTERED П-3 POLYUNSATURATED FATTY ACIDS AND SIMVASTATIN ON THE METABOLIC DISTURBANCES

Victoriya Serhiyenko[1], O. Serhiyenko[1], L. Serhiyenko[2]

[1]Endocrinology, [2]Medical Biology, National Medical University, Lviv, Ukraine

The aim of study was to examined the effects of simvastatin (SIM) and ϖ-3 polyunsaturated fatty acids (ϖ-3 PUFA) combinations in Type 2 diabetic patients with diabetic cardiomyopathy (DCMP).

Material and methods: 61 patients with Type 2 diabetes mellitus (DM) and DCMP were allocated to three groups. Patients of group A (n=22) received SIM 20 mg tid; B (n=21) - capsules of fish oil (1,0 g eicosapentaenoic, 1,0 g docosahexaenoic acid) and 0,1% α-tocopherol acetate tid; C (n=18) - SIM 10 mg tid plus capsules of fish oil. The duration of the study was 2 months. We investigated circulating of insulin (IRI), C-peptide, leptin, high sensitivity C-reactive protein (hs-CRP), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), total cholesterol (TC), low (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG) concentrations in the blood plasma. HOMA-IR and HOMA-β-cell function (HOMA-β-CF) indexes were calculated. Statistics: ANOVA.

Results: Lipid disorders in the patients with DCMP [high level of total TC, LDL-C, TG and decrease level of HDL-C], increase of circulation IRI, C-peptide, leptin, hs-CRP, IL-8, TNF-α concentration in blood plasma, indexes of HOMA-IR and decrease of HOMA-β-CF parameters were found. After 2 months of treatment a more significant decrease of IRI (19,67±2,73 mU/ml), HOMA - IR ($p < 0,001$), leptin, C-peptide [1115,6±123,9 pM/l ($p < 0,001$)], increase of HOMA-β-CF parameters were observed in the 3-rd group.

Conclusions: Usage of SIM and ϖ-3-PUFA is accompanied by significant improvement of the lipid profile, other biochemical parameters, that allows to recommend their combination in the treatment of patients with DCMP.

Memory Stick

THE EFFECTS OF COMBINED ATORVASTATIN AND EXERCISE TREATMENT ON THE DEVELOPMENT AND STABILITY OF THE ATHEROSCLEROTIC PLAQUES - EXPERIMENTAL STUDY

Petros Moustardas[1], N. Kadoglou[1], M. Katsimpoulas[1], A. Kapelouzou[1], N. Kostomitsopoulos[1], P. Karayannakos[1], C. Liapis[2]

[1]Center of Experimental Surgery, Biomedical Research Foundation Academy of Athens, [2]Department of Vascular Surgery, 'Attikon' Hospital, Medical School, University of Athens, Athens, Greece

Objectives: The present study investigated the effects of combined atorvastatin and exercise treatment on the stability of atherosclerotic plaques in apoE knockout (apoE-/-) mice.

Methods: Forty male, apoE-/- mice were fed a western-type high-fat diet for 16 weeks. Thereafter, they were randomized into four (n=10) groups for 8 additional weeks. A)Normal diet: Control group. B)Normal diet+atorvastatin administration (esophageal catheter, 10mg/Kg/day) C)Normal diet+exercise program on treadmill (5/week,60min/session). D)Normal diet+atorvastatin+exercise. At the study's end, all mice were sacrificed. Atherosclerotic lesions of the aortic arch were quantitatively examined. Mean lumen stenosis ratio and the relative concentrations of collagen, elastin, MMP8 and TIMP-1 within plaques were determined (one-way ANOVA).

Results: Aortic lumen stenosis ratio was significantly reduced in groups B (49.5±13.77%,$p< 0.001$), C (56.1±4.76%,$p=0.020$) and D (45.67±5.15%,$p< 0.001$) compared to control group (67±9.49%). Comparative evaluation revealed increase in collagen and elastin concentrations in all active groups compared to group A ($p< 0.001$). In post-hoc analysis group D showed the most pronounced increment in collagen (35.22±7.03%) and elastin (24.99±2.52%) concentrations within the atherosclerotic lesions compared to B (21.9±9.7%, 18.63±4.8%, respectively) and C groups (20.41±3.21%, 16.84±0.79%, respectively) ($p< 0.05$). Finally, MMP-8 relative plaque concentration was considerably lower in groups B, C and D compared to group A ($p< 0.05$), with TIMP-1 following the inverse pattern ($p< 0.05$). Notably, body-weight and lipid parameters didn´t differ between groups at the end ($p>0.05$).

Conclusion: Combined atorvastatin and exercise treatment of $apoE^{-/-}$ mice regressed atherosclerotic lesions and remarkably improved their histologically measured stability, more than each intervention alone.

Memory Stick

EFFECTS OF DALCETRAPIB IN COMBINATION WITH PRAVASTATIN ON LIPOPROTEIN PROFILE IN PATIENTS WITH TYPE II DYSLIPIDAEMIA

Michael Miller[1], C.M. Ballantyne[2], E.J. Niesor[3], T. Burgess[4], D. Kallend[3], E.A. Stein[5]

[1]University of Maryland Medical Center, Baltimore, MD, [2]Baylor College of Medicine, and Methodist DeBakey Heart and Vascular Center, Houston, TX, USA, [3]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [4]Hoffmann-La Roche Inc., Nutley, NJ, [5]Metabolic and Atherosclerosis Research Center, Cincinnati, OH, USA

Background: Dalcetrapib, an agent that reduces CETP activity, raised HDL cholesterol levels by 17%, 31% and 36% (300, 600 and 900 mg, respectively) in a phase IIb trial in hyperlipidaemic patients (HDL < 50 mg/dL) receiving 40 mg/day pravastatin (n=292). Here we present lipoprotein subfraction data from the same study.

Methods: Patients received pravastatin plus either placebo or 300/600/900 mg/day dalcetrapib for 12 weeks. Lipoprotein subclasses were measured at baseline and week 12 using nuclear magnetic resonance (NMR) and gradient gel electrophoresis.

Results: At 12 weeks, NMR and electrophoresis revealed a shift in HDL and LDL lipoprotein profiles in all treatment arms (Table).

Secondary efficacy parameter measured by NMR (N) or electrophoresis (E)	**% change from baseline to week 12 (600 mg dalcetrapib)**
HDL particles (total) (N)	+10.9
Large HDL (N)	+171.3
Medium HDL (N)	+69.6
Small HDL (N)	+2.4
HDL 2a (E)	+10.1
HDL 2b (E)	+30.1
HDL 3a (E)	-6.7
HDL 3b (E)	-13.9
HDL 3c (E)	-14.6

[Effect of dalcetrapib 600 mg on HDL profile (n=67)]

The percentage of large HDL particles increased with dalcetrapib dose. ApoE was unchanged in total plasma but was increased in the HDL fraction.

Conclusions: Dalcetrapib in combination with pravastatin improved the atherogenic lipoprotein profile in dyslipidaemic patients.

Memory Stick

REDUCTION OF EXISTING ATHEROSCLEROTIC PLAQUES: LIPID-LOWERING AND ANTI-INFLAMMATORY STRATEGIES PROMOTE LESION REGRESSION IN APOE3LEIDEN TRANSGENIC MICE

P.Y. Wielinga[1], **Robert Kleemann**[1], J. de Vries-vd Weij[1], K. Toet[1], P.C.N. Rensen[2], L. Verschuren[1], T. Kooistra[1]

[1]TNO BioSciences, [2]LUMC, Leiden, The Netherlands

Atherosclerosis was previously thought to be a disease primarily involving lipid accumulation in the arterial wall. Current concepts of the disease include involvement of the immune system and chronic inflammation as crucial factors in all stages of the atherosclerotic process. The central role of inflammation in atherogenesis suggests that anti-inflammatory therapies might have a beneficial role in management of already existing, manifest atherosclerotic lesions.

Using a humanized and drug-sensitive animal model that allows studying atherosclerotic lesion regression, ApoE3Leiden mice, evidence is provided for a health benefit by quenching the inflammatory component of the disease. Compounds with anti-inflammatory CRP-lowering properties, salicylate and activators of the Liver X receptor (LXR), were tested.

Salicylate treatment reduces atherosclerotic macrophage content and increases lesion stability of pre-existing plaques through quenching of NF-κB activity and reduction of plasma cholesterol. Likewise, pharmacological activation of LXR reduces the inflammatory state and promotes lesion regression regarding lesion number, area, and severity. More specifically, LXR activation induces lesional macrophage disappearance and increases the expression of the chemokine receptor CCR7, a factor functionally required for regression.

Memory Stick

A CLINICAL STUDY OF CHOLESTEROL-INDEPENDENT EFFECT EFFECTS OF STATIN THERAPY ON VASCULAR INFLAMMATION: STATINS SELECTIVELY AND DOSE-DEPENDENTLY ATTENUATE MACROPHAGE ACTIVATION

Jan H N Lindeman

LUMC, Leiden, The Netherlands

Inflammation plays a key role in the initiation and progression of atherosclerotic disease. Independent of their lipid lowering effects statins have been reported to reduce (vascular) inflammation, but the relevance of this effect for human disease remains unclear. In this study we evaluated the effect of intermediate doses (20/40 mg) Simvastatin or Atorvastatin on vascular inflammation in the abdominal aortic aneurysm; a pathology that belongs to the atherosclerotic spectrum of diseases, but that is not cholesterol driven.

A comprehensive analysis using immunohistochemistry, mRNA and protein analysis, and protease assays showed that statins do not influence the cellular content of the aneurysmal wall. Yet, we did observe a dose dependent and selective reduction of the cytokines IL-6 and MCP-1 in the aortic wall. Further analysis showed that these changes on the chemokine/cytokine were followed by a reduction in the macrophage activation markers (Cathepsin K and ALOX5), but not other macrophage markers such as MMP12 or COX2. Statin treatment did not influence the activation status of other cell types such as T-helper, cytotoxic T-cells, B cells/plasma cells or neutrophils.

Our studies show that, independent of their lipid lowering effect, statins exhibit a dose dependent and highly selective effect on vascular inflammation, an effect that is presumably mediated by an effect on NFkB activity. The changes on chemokine/cytokine level are followed by a reduced activation of selected macrophage subsets but not of other cell types. The selective effect on vessel wall IL-6 expression may explain the reduced CRP levels upon statin therapy.

Memory Stick

ECHOCARDIOGRAPHIC VISUAL VERSUS COMPUTERIZED EVALUATION OF THE DEGREE OF AORTIC VALVE CALCIFICATION IN PATIENTS WITH AORTIC VALVE AND ROOT PATHOLOGY

Mohamed Yousry[1,2], M.J. Eriksson[1], A. Rickenlund[1], J. Petrini[1], T. Gustavsson[3], P. Eriksson[1], A. Hamsten[1], A. Franco-Cereceda[1], J. Liska[1], K. Caidahl[1]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Suez Canal University, Ismailia, Egypt, [3]Chalmers School of Technology, Göteborg, Sweden

Aortic valve calcification (AVC) is considered a part of generalized atherosclerosis and has prognostic importance in general population. However, its classification is subjective and requires training. Quantitative measure of valve-echogenicity by computerized grey-scale analysis (GSA) of images from transthoracic (TTE) or transesophageal (TEE) echocardiography might be an objective alternative.

Aims: To determine whether GSA improves evaluation of AVC in aortic valve (AV) or aortic-root pathology in comparison to visual evaluation of TTE and TEE real-time images, using intraoperative evaluation as a golden standard.

Methods: Two-dimensional short-axis view (2D, SAX) recordings of the AV were obtained by TTE and TEE in 175 patients at surgery for aortic stenosis (n=110), aortic regurgitation (n=60) or aortic-root dilatation (n=5). Visual evaluation was performed in real-time sequences by a 5-degree scale, also applied intraoperatively by the surgeon. The same visual scale was applied on end-diastolic TTE and TEE stop-frames in order to evaluate the importance of real-time images in evaluation. GSA was independently applied at the same end-diastolic stop-frames.

Results: Correlations between ultrasonic and intra-operative evaluations of AVC are given in Table1.

Conclusions: Visual evaluation of AVC in ultrasonic real-time sequences was closer related than evaluation in stop-frames to the intraoperative score. In real-time but not in stop-images, TEE was superior to TTE. GSA was not superior to visual assessment of stop-frames, and further development of GSA software also for real-time images might add diagnostic accuracy.

	TTE-GSA	TTE-Real-time-evaluation	TTE-stop-images evaluation	TEE-GSA	TEE Real-time evaluation	TEE-stop-images evaluation
Intraoperative-evaluation	r=0.61**	r=0.74**	r=0.63**	r=0.49**	r=0.81**	r=0.65**

[Summary of Results]

Memory Stick

DEVELOPMENT AND CHARACTERIZATION OF A SHAPE-MEMORY POLYMER-BASED CORONARY STENT WITH ENDOTHELIAL PROGENITOR CELL-ADHESIVE CAPACITY

Felix Vogt[1], M. Borinski[1], F. Schreiber[2], C. Flege[1], N. Krott[1], T. Gries[2], C. Weber[3], R. Hoffmann[1], N. Marx[1], R. Blindt[1]

[1]Cardiology, Pneumology and Angiology, University Hospital, RWTH Aachen, [2]Institute for Textile Technology, RWTH Aachen University, [3]Institute for Molecular Cardiovascular Research, University Hospital, RWTH Aachen, Aachen, Germany

Objective: Shape-memory polymers (SMP) have been advocated for medical applications; endothelial progenitor cell (EPC) recruitment to sites of coronary injury has been shown to accelerate reendothelialization and to reduce ISR. The aim of the present study was to develop processing methods and to characterize mechanical, biocompatibility, and EPC-adhesive properties of a novel coronary polyurethane-based SMP stent.

Methods: By a newly developed, three-dimensional braiding machine, polyurethane was melted, shaped to monofilaments, and processed to stents with adjustable properties. Mechanical testings for radial and tensile strength were performed. To assess SMP biocompatibility, during direct and indirect contact of EPC, human umbilical vein endothelial cells, or smooth muscle cells to SMP, viable or necrotic cells and proliferative or migratory activity were determined. EPC attachment and outgrowth were evaluated by a laminar flow chamber assay and by scanning electron microscopy.

Results: By adaptation of monofilament and stent diameter, number of monofilaments, monofilament draw ratio, ankle, and heat treatment, tailored production of coronary stents was feasible. By heat treatment at 125°C, radial strength of SMP stents was increased and comparable to nitinol stents. SMP biocompatibility assessment evidenced no reduction of cellular vitality or migratory and proliferatory activity. EPC displayed pronounced adhesive capacities and did adopt an endothelial phenotype.

Conclusion: SMP can be processed to tailored coronary stents that display appropriate radial strength, a pertinent biocompatibility pattern, and potentially beneficial EPC-adhesive capacities. SMP combined with the novel manufacturing technique might represent an interesting coronary stent development strategy potentially reducing ISR or in-stent thrombosis by accelerated reendothelialization.

Memory Stick

IMMUNOSUPPRESSIVE AND ANTI-DYSLIPIDEMIC TREATMENT AFTER KIDNEY AND KIDNEY-PANCREAS TRANSPLANTATION

Tomislav Bulum, I. Prkačin, D. Vujanić, M. Knotek

Department of Nephrology, University Hospital Merkur, Zagreb, Croatia

Introduction: Hyperlipidemia is a frequent finding among renal transplant recipients. Treatment of dyslipidemias should be part of routine post-renal transplant care. Immunosuppressive agents, particularly corticosteroids, calcineurin inhibitors and rapamycin, have dose-related effects on serum lipid levels. We investigated the frequency of anti-dyslipidemic drugs in relation to immunosuppressive agents.

Patients and methods: 163 patients (median age 44, range 18-70 years, 98M/65F, 98 with kidney transplant (KT) and 64 with kidney and pancreas transplant (SKPT)) have been evaluated for taking of immunosuppressive agents and treatment of dyslipidemia.

Results: Seventy-eight (47%) of transplanted patients take lipid lowering drugs, 71 of them (91%) take fluvastatin, 7 patients (9%) atorvastatin. Fifty-three patients with KT (54%) and 25 patients with SPKT (39%) take antilipemic agents. Corticosteroids take 132 of all patients (80.9%), while in 50% of those lipid lowering drugs were introduced. Cyclosporine take 37.4% of all patients, in 55.7% of those lipid lowering drugs were introduced. Rapamycin take only 6 (0.03%) of all patients, in 3 (50%) of them lipid lowering drugs were introduced. Much better lipid profile was on tacrolimus therapy.

Conclusion: The presented results show a high incidence of hyperlipidemia in patients with KT and SPKT, which is in line with results from other published studies. The largest number of transplanted patients was on corticosteroid therapy. The most frequent used anti-dyslipidemic drug was fluvastatin according to the largest experience (ALERT study). It is recommended to replace cyclosporine by tacrolimus, to discontinue sirolimus if dyslipidemia occurs, and continue low-dose prednisone.

Memory Stick

EFFECT OF NIACIN WITH ATORVASTATIN ON SECRETORY PHOSPHOLIPASE A2 IN MEN WITH CORONARY HEART DISEASE AND LIPOPROTEIN(A) EXCESS

E. Trukhacheva, **Marat Ezhov**, V. Titov, O. Afanasieva, M. Afanasieva, A. Lyakishev, V. Kukharchuk, S. Pokrovsky

Federal State Institution " Russian Cardiology Research - and Production Complex Rosmedtekhnologies", Moscow, Russia

Objective: To evaluate the effect of atorvastatin alone and in combination with niacin on level of secretory phospholipase A2 (sPLA2) in men with coronary heart disease (CHD) and lipoprotein(a) [Lp(a)] excess.

Methods: We performed randomised controlled study in 60 men (mean age 54 years) with angiographically documented CHD and Lp(a) ≥ 30 mg/dl. Patients received either atorvastatin 10-20 mg/day (Group I, n=30) or its combination with niacin 2.0 g/day (Group II, n=30). We have measured the serum levels of total cholesterol (TC), triglycerides (TG), HDL-C, Lp(a), high sensitivity C-reactive protein (hsCRP), sPLA2 at baseline and after 6 months of therapy.

Results: Before enter the study, the most of patients took statins: 90% in Group I and 60% in Group II. All baseline characteristics were comparable between groups. In 6 months there was significant decreasing of concentrations of TC (5.0±1.1 to 4.1±0.7 mmol/l, $p < 0.05$), LDL-C (3.0±1.1 to 2.2±0.5 mmol/l, $p < 0.05$), and Lp(a) (90±10 to 66±13 mg/dl, $p < 0.05$) in Group II but not in Group I. We obtained lowering of sPLA2 levels both in Group I (642±306 to 415±150 ng/ml, $p < 0.05$) and Group II (520±310 to 350±221 ng/ml, $p < 0.05$) and, importantly, in each patient. Concentrations of hsCRP remained unchanged during the study in both groups.

Conclusion: In men with coronary heart disease and Lp(a) excess combination of atorvastatin with niacin significantly lowered levels of secretory phospholipase A2 and Lp(a).

Memory Stick

EFFECT OF EVEROLIMUS ON PRE-EXISTING ATHEROSCLEROSIS IN LDLR-/- MICE

Désiré Brendel, F. Beutner, D. Teupser, M. Müller, R. Baber, F. Jeromin, W. Wilfert, U. Ceglarek, J. Thiery

Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Leipzig, Leipzig, Germany

Objective: Inflammatory processes and proliferation play a critical role in atherogenesis. Proliferation signal inhibitors rapamycin and everolimus has been shown to decrease de novo development of atherosclerosis in different mouse models. However, their effect on pre-existing atherosclerosis has not been studied yet. Thus, the aim of the present study was to investigate the effect of everolimus on hypercholesterolemia-induced atherosclerosis in LDL-receptor-deficient (LDLR-/-) mice.

Methods and results: Atherosclerosis development was induced by a 0.1% cholesterol diet over a period of 12 weeks. For regression studies, diet was changed to 0.0% cholesterol and mice were assigned to three experimental groups (n=60) containing either no everolimus (control), 7.5mg everolimus (low dose, LD) or 37.5mg everolimus (high dose, HD) per kg diet for 12 weeks. Treatment resulted in dose-dependent everolimus blood levels of 36±24µg/l (LD) and 180±100µg/l (HD). Statistical analysis of morphometric quantification assumed a significant reduction of atherosclerotic lesion size after adjustment for cholesterol. In fact, the effect vanished because of a drug-induced hypercholesterolemia. HD mice had 40% higher levels of plasma cholesterol caused by an increase of cholesterol in LDL and VLDL fractions. Absolute atherosclerotic lesion size was decreased by 25% in LD and HD, compared to control, however, the reduction did not reach statistical significance.

Conclusion: While previous studies showed a strong inhibition of de-novo development of atherosclerosis, our current results demonstrate that everolimus does not lead to regression of pre-existing atherosclerosis in LDL-/- mice. Our findings suggest that everolimus might exert more potent anti-atherogenic properties in earlier stages of atherogenesis

Memory Stick

CAROTID ULTRASOUND CONTRIBUTES TO THE ASSESSMENT OF CORONARY ARTERY DISEASE

Frank Beutner[1,2], D. Teupser[2], S. Gielen[1], M. Sandri[1], M. Scholz[3], G. Schuler[1], J. Thiery[2]

[1]Department of Cardiology, Heart Center, University Leipzig, [2]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, [3]Institute of Medical Informatics, Statistics and Epidemiolgy (IMISE), University Leipzig, Leipzig, Germany

Objective: There is evidence that non-invasive vascular imaging is useful for risk prediction of cardiovascular morbidity and mortality. However there is limited data concerning the diagnostic value of carotid ultrasound in patients with suspected coronary artery disease (CAD). The aim of this study was to analyze carotid intima-media thickness (cIMT) and the extent of carotid plaque presence in angiographically assessed patients.

Methods and results: Patients undergoing coronary angiography for suspected CAD (Leipzig Heart Study) were sonographically screened for atherosclerotic plaque in the carotid arteries and carotid plaque score (cPS) was determined. cIMT of the common carotid arteries was analyzed using a semi-automated IMT detection algorithm. 10-year CHD risk was calculated using the Framingham Risk Score (FRS). Analyses were performed for sets of patients with normal angiogram (n= 547), non-obstructive CAD (n= 288) and obstructive CAD (n= 637) showing at least one >50% stenosis in a major coronary artery. CAD patients had significant higher levels of FRS (10.9 ± 8.1% vs. 17.1 ± 10.6%), cPS (0.6 ± 1.0 vs. 1.8 ± 1.4) and cIMT (0.75 ± 0.14 vs. 0.81 ± 0.14mm). In ROC analysis cPS (AUC 0.75) reached the highest predictive value while cIMT (AUC 0.62) and FRS (AUC 0.65) showed only weaker predictive values.

Conclusion: Carotid plaque presence is a strong predictor for CAD with more valuable impact than cIMT and conventional risk profile. Automation and advanced technical tools in ultrasonic imaging for focal plaque in subclinical stages will improve the prediction of CAD.

Memory Stick

DIRECT COMPARISON OF ANGIOGENIC GENE THERAPY AND STEM CELL THERAPY IN PATIENTS WITH CHRONIC LIMB ISCHEMIA

Konstantin Talitskiy, O. Bulkina, T. Arefieva, O. Vorobieva, T. Balakhonova, I. Levitskiy, D. Filatov, A. Eliseev, V. Kukharchuk, O. Stukalova, A. Fedorovich, Y. Parfyonova, Y. Karpov

Russian Cardiology Research Centre, Moscow, Russia

Therapeutic angiogenesis is a new promising treatment strategy for patients with chronic limb ischemia who are not candidates for interventional treatment. Previous studies show improvement after stem cell (SC) or angiogenic gene therapy, but there is lack of studies comparing both therapeutic approaches. 35 patients with Fontaine IIb-IV atherosclerotic limb ischemia were randomized for VEGF gene therapy (GT), SC therapy or conventional treatment (CT). GT group (n=20) received 2 or 3 sets of intramuscular injections with VEGF-plasmid. SC group patients (n=5) received local injections of autologous G-CSF-stimulated peripheral blood mononuclear cells. 3 months after treatment GT and SC groups show significant improvement of walking distance, everyday activity and ankle-brachial index (ABI). Improvement of walking distance was more prominent in GT than in SC group (3.22 vs 2.09 times, respectively) whereas ABI enhancement was better in SC group (12% vs 50%). Both groups show increase of limb perfusion assessed by 99mTc-MIBI scintigraphy, contrast MRI, laser Doppler and transcutaneous oxymetry. Marked increase of collateral network was observed angiographically in about 40% of GT and SC patients. No serious complications were observed. An ischemic ulcer had successfully healed in SC patient 1 month after treatment. No significant change of clinical or laboratory indices were found in CT group patients (n=10). Both GT and SC groups show significant increase of CD45-CD34+AC133+ circulating progenitor cell number after treatment, with more prominent elevation in SC group, which correlates with limb perfusion and improvement of ischemic symptoms.

Memory Stick

DIFFERENTIAL EFFECTS OF STATINS ON VITAMIN D METABOLITES: IMPLICATIONS FOR INSULIN SENSITIVITY

Jerzy Beltowski

Dept. of Pathophysiology, Medical University, Lublin, Poland

The effect of statins on insulin sensitivity is controversial. Whereas pravastatin has been demonstrated to reduce the incidence of diabetes, several synthetic statins may impair insulin sensitivity. An active form of vitamin D, 1,25-dihydroxycholecalciferol (calcitriol, $1,25(OH)_2D_3$) is involved in the regulation of insulin secretion and action. We examined the effect of statins on vitamin D metabolites and insulin sensitivity in the rat. Male Wistar rats were treated with either pravastatin or atorvastatin (20 mg/kg/day) for 3 weeks. Pravastatin increased 25-hydroxycholecalciferol ($25(OH)D_3$) and $1,25(OH)_2D_3$ concentrations whereas atorvastatin reduced $25(OH)D_3$ but had no effect on $1,25(OH)_2D_3$. Pravastatin increased insulin sensitivity (QUICKI index) whereas atorvastatin had the opposite effect. Effect of pravastatin on insulin sensitivity was mimicked by administration of exogenous $1,25(OH)_2D_3$. In contrast, detrimental effect of atorvastatin on insulin sensitivity was not abolished by concomitant administration of exogenous vitamin D_3 at doses which restored plasma $25(OH)D_3$ level. Coadministration of mevalonate prevented the effect of pravastatin on $25(OH)D_3$ and $1,25(OH)_2D_3$ but did not prevent the reducing effect of atorvastatin on $25(OH)D_3$. In conclusion:

(1) pravastatin and atorvastatin have opposite effects on vitamin D metabolites and insulin sensitivity,

(2) pravastatin improves insulin sensitivity by increasing $1,25(OH)_2D_3$ concentration,

(3) detrimental effect of atorvastatin on insulin sensitivity is independent of its reducing influence on $25(OH)D_3$,

(4) positive effects of pravastatin on vitamin D metabolites and insulin sensitivity result from the inhibition of HMG-CoA reductase,

(5) reducing effect of atorvastatin on $25(OH)D_3$ is independent of HMG-CoA reductase blockade.

Memory Stick

COULD PLASMA LEVEL OF ASYMMETRIC DIMETILARJININ BE A MARKER FOR STENT RESTENOSIS?

Ugur Bal[1], S. Kanyılmaz[1], A. Yıldırır[1], A. Aydınalp[1], G. Kaynar[1], M. Koza[2], H. Muderrisoglu[1]

[1]Cardiology, [2]Başkent University Hospital, Ankara, Turkey

Introduction: Neointimal hyperplasia consisting of smooth muscle cells and matrix is the main reason for stent restenosis.Nitric oxide could preclude the neointimal hyperplasia by inhibiting smooth muscle cell proliferation. Asymmetric Dimetilarjinin (ADMA) is competitive inhibitor of nitric oxide synthetase. The aim of this study is to investigate whether the level of plasma ADMA is a marker for stent restenosis or not.

Material and method: In this study we assigned 91 patients previously known coronary stent. Plasma ADMA levels measured by high-performance liquid chromatography (HPLC).The clinical and laboratory data compared between patients with and without restenosis.

Results: 144 stents of 91 patients evaluated. In 35 patients (38.5%) and 46 (31.9%) of 144 stents angiographic stenosis was found. Plasma ADMA levels (0.50 ± 0.18µmol / L and 0.39 ± 0.11µmol / L, p = 0.001) and stent length (16.05 ± 5.14mm and 14:27 ± 4.13mm, p = 0.047) were higher however; left ventricular ejection fraction (49.6% ± 10.4% and 54.1 ± 8.2, p = 0.023) and stent diameter (2.81 ± 0.33 and 3:00 ± 0.39, p = 0.018) were lower in patients who had restenosis, as compared to patients who had not. In multiple linear regression analysis, plasma ADMA level (β = 0281, p = 0.012), stent diameter (β = -0302, p = 0.001) and stent length (β = 0238 p = 0.015) were found as independent markers of restenosis.

Conclusion: Plasma ADMA levels may be markers for stent restenosis, but should be evaluated in larger clinical studies.

Memory Stick

ELEVATION OF INTERLEUKIN 6 ASSOCIATED REDUCTION OF LIPOPROTEIN(A) PREDICTS RESTENOSIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION PERFORMED SUCCESSFUL PRIMARY PTCA

Akihisa Fujino[1], T. Watanabe[2], K. Taira[2], K. Ashikawa[2]

[1]Cardiology, [2]Yonezawacity Hospital, Yonezawa, Japan

Object: To examine a relation between serial change of serum interleukin6 (IL6) and serial change of lipoprotein(a) (Lp(a)) and examine this relation with restenosis in patients with AMI performed successful primary PTCA with bare metal stent.

Method: We examined serial change of serum IL6 and Lp(a) in patients with AMI from January 2003 to August 2008 in 154 cases among 256 consequent AMI. The objects were 102 male and 52 female, and mean age was 69.5±8.4. Restenosis is defined as 50% or more stenosis evaluated by QCA-CMS at follow up CAG.

Result: 31 cases showed restenosis (group R) and 123 cases showed no restenosis (group S). In group R IL6 on admission day was 26.8±34.2 and was on the third day 168.2±12.3 (pg/ml) (mean±SD) In group S IL6 admission day was 16.3±12.8 and was on the third day 31.6±34.8. IL6 on third day was significantly higher in group R ($p < 0.0001$ Wilcoxon's test). In group R Lp(a) was 30.9±18.1 (mg/dl) (mean±SD) admission day and was 24.3±16.2 on third day. In group S Lp(a) was 27.9±19.6 admission day and was 35.4±21.3 on third day. Lp(a) was significantly lower in group R ($p < 0.001$ Wilcoxson's test).

Conclusion: Serial measurement of serum IL6 and Lp(a) would predict restenosis in patients with AMI performed successful primary PTCA with bare metal stent.

Memory Stick

EFFECT OF SLCO1B1*5 (VAL174ALA) ALLELE ON LIPID LOWERING RESPONSE OF ATORVASTATIN IN THE 4D STUDY

Michael M. Hoffmann[1], K. Winkler[1], C. Drechsler[2], V. Krane[2], W. März[3,4], C. Wanner[2]

[1]Division of Clinical Chemistry, Department of Medicine, University Medical Center, Freiburg im Breisgau, [2]Division of Nephrology, Department of Medicine, University Hospital, Würzburg, [3]Department of Public Health, Social and Preventive Medicine, Mannheim Medical Faculty, University of Heidelberg, Mannheim, [4]Synlab Center of Laboratory Diagnostics, Heidelberg, Germany

Aims: The range of individual responses to statin therapy is broad and data from several studies suggest that genetic factors play an important role in the determination of this response. We therefore analysed a common variant (Val174Ala, 521T-C, rs4149056) in the hepatic drug transporter *SLCO1B1* in participants of the 4D (German Diabetes and Dialysis Study) trial.

Methods: The 4D-study was a randomized trial including 1255 patients with type 2 diabetes mellitus and previous duration of haemodialysis of less than two years. The patients were randomly assigned to double-blind treatment with either 20 mg of atorvastatin (n=619) or placebo (n=636) once daily. Lipids were analysed at baseline and after 6 months. DNA of 1177 patients was available for analysis. SLCO1B1 genotypes were analysed by PCR and subsequent RFLP.

Results: In the 4D study population the allele frequency for SCLO1B1*5 was 17.7 %, genotypes were equally distributed between cases and controls. At randomization the median level of LDL-C was 124 mg/dl, after 6 months in the atorvastatin group the median level was 74 mg/dl (- 41 %) and in the placebo group 120 mg/dl (-3.6 %). The SCLO1B1*5 allele influenced LDL-C levels neither at baseline nor under therapy. The same was due to other lipid parameters.

Conclusions: In contrast to previous published results that SCLO1B1*5 is leading to a greater statin concentration we did not found any association between the SCLO1B1*5 allele and the LDL-C lowering effect of atorvastatin.

Memory Stick

INTRAINDIVIDUAL COMPARISON OF WHOLE BODY MR ANGIOGRAPHY AT 1.5 AND 3 TESLA IN HIGH RISK PATIENTS WITH HEREDITARY HYPERLIPIDEMIA

P. Bannas[1], A.-K. Fink-Wedel[1], **Jan-Hendrik Buhk**[1], H. Kooijmann[2], F.-U. Beil[3], T. Bley[1], G. Adam[1], C. Weber[1]

[1]Radiology, University Hospital Hamburg-Eppendorf, [2]Philips Medical Systems, Hamburg, Germany, [3]Internal Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Purpose: To prospectively and intraindividually compare image quality and depiction of defined anatomic vascular regions in contrast enhanced whole-body MRA (WBMRA) at 1.5 and 3 Tesla in patients with hereditary hyperlipidemia, which is associated with a high risk of premature cardiovascular disease and death.

Material and methods: 27 patients (18male, 9female, mean age 56years, ranging from 30-68 years) with hereditary hyperlipidemia (Apo E2/2, Apo B3500) received a 1.5 and 3 Tesla gadobutrol contrast enhanced WBMRA scan within 14 days. Two experienced radiologists evaluated WBMRA scans in consensus regarding depiction of target vessels, image quality according to a 5-point-scale (not evaluable to excellent), degree of stenosis (0-24%, 25-49%, 50-74%, 75-99%, 100%) as well as vessel alterations such as aneurysms.

Results: 1.5 Tesla and 3 Tesla scans yielded high-quality MR angiograms in all patients. Image quality at 3.0 Tesla was rated superior (4.1+/-0.7) to image quality at 1.5 Tesla (3.8+/-0.9), however without proven statistically difference. In 20 patients (74%) there was conformity between 1.5 and 3 Tesla regarding the grade of stenosis. All stenoses greater than 50% (n=6), occlusions (n=6) and aneurysms (n=3) were evaluated similarly at both field strengths in 8 patients. Low-grade stenoses in the peripheral parts of the arteries were detected more often at 3 Tesla. In 8 patients additional unsuspected anomalies were found.

Conclusion: WBMRA resulted in diagnostic image quality at 1.5 and 3 Tesla in all patients. Imaging at 3 Tesla revealed better diagnostic performance in detection of peripheral stenoses.

Memory Stick

COMPARISON OF 64-SLICE MDCT AND INVASIVE ANGIOGRAPHY IN DIAGNOSIS OF SIGNIFICANT CORONARY ARTERY STENOSIS

Reza Javadrashid[1], A. Salehi[1], M. Daghigi[1], M. Tarzamni[1], N. Aslanabadi[2]

[1]Radiology, [2]Cardiology, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Introduction of 64-Slice Multidetector Computed Tomography (MDCT (has resulted in a great improvement in the non-invasive coronary artery imaging.

Methods: In a retrospective cross sectional study we evaluated the diagnostic accuracy of 64-Slice MDCT versus invasive coronary angiography in patients with the suspected coronary artery disease who underwent both MDCT and invasive coronary angiography (ICA). Computed tomography angiography and invasive coronary angiography findings of each coronary segment were compared to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MDCT in the detection of significant lesions(stenosis).

Results: A total of 125 patients were evaluated with both methods. In per patient assessment, the sensitivity, specificity, PPV and NPV of MDCT were 97.6%, 85.2%, 95.2% and 92% respectively. These values in per vessel evaluation were 86.3%, 92.2%, 86.8%, and 92.2%, and also corresponding values in per segment evaluation were 69.8%, 94.8%, 69%, and 95% respectively.

Conclusion: We concluded that coronary angiography with 64-Slice MDCT has high diagnostic performance for evaluation of significant coronary artery disease (CAD) in patients with the suspected coronary artery disease but cannot replace conventional coronary angiography at present time.

Keywords: Significant coronary artery disease, invasive coronary angiography (ICA), 64 Slice MDCT

Memory Stick

FOLLOW-UP OF CHILDREN AFTER POSITIVE GENETIC TESTING IN THE DUTCH NATIONAL SCREENING PROGRAM FOR FAMILIAL HYPERCHOLESTEROLEMIA

Hans J Avis[1], R. Huijgen[1], B.A. Hutten[2], M.N. Vissers[1], T.H. Janssen[3], D.M. Kusters[1], I. Kindt[4], J.J. Kastelein[1], F.A. Wijburg[5]

[1]Department of Vascular Medicine, [2]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, [3]Medical Student, Academic Medical Center, University of Amsterdam, [4]Stichting Opsporing Erfelijke Hypercholesterolemie (StOEH), [5]Department of Metabolic Diseases, Emma Childrens Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction: Like in several countries, a nationwide genetic screening program is active in the Netherlands that, on parents' request, tests children for familial hypercholesterolemia (FH). Although treatment guidelines for FH children have been published, the follow-up of these children is unknown. Our aim was to assess current clinical practice and follow-up of children diagnosed in the Dutch screening program.

Methods: We sent questionnaires to parents of FH patients (age 0-18), 1.5 years after diagnosis. The questionnaire included questions on demographics, family history, doctor consultation, and treatment. We aim to retrieve 200 questionnaires by December 2009.

Preliminary results: Hitherto, we retrieved 152 questionnaires concerning 67 boys and 85 girls, with mean age at diagnosis 10.6±4.2 (SD) years and mean LDL-C at diagnosis 4.31±1.26 mmol/l. Of these, 120 (79%) patients consulted a physician: general practitioner (38%), lipid-clinic specialist (25%), paediatrician (22%), internist (11%), and other (4%). Due to referral, eventually 18%, 34%, 26%, 14% and 8% consulted abovementioned physicians, respectively. LDL-C at diagnosis and family history for cardiovascular disease revealed to be independent predictors of physician consultation. Of 35 children aged 8-14 with an LDL-C >4.0 mmol/l, 15 (43%) were on statin treatment. Of 37 children aged >14 with an LDL-C >3.5 mmol/l, this number was 10 (27%). Independent predictors for medication use were age and LDL-C at diagnosis.

Conclusion: Although a physician is consulted in most cases, a considerable number of children remain untreated, despite high LDL-C levels. Improved education of patients, parents, and physicians may improve management of FH children.

Memory Stick

OXIDATIVE STRESS PARAMETERS IN HDL-LIPOPROTEIN FRACTION IN CHANGED PHYSIOLOGICAL STATES

Ivana Srećković

Institut for Medical Biochemistry, Belgrade University, Belgrade, Serbia

Introduction: Many experimental researches indicate that oxidative stress is associated with physiological states followed by a high-energy demand and an increased oxygen requirement. Intensive oxygen metabolism imposes a potential threat to cells because of the formation of reactive oxygen species which lead to oxidative damage.

The aim of study: The aim of this study was to estabilish existence and influence of oxidative stress to paraoxonase activity in HDL-lipoprotein fraction.

Material and methods: The research focused on 48 pregnant women (falowed during pregnancy and after childbirth in 5 time points), 15 sports players and 30 healthy individuals. As markers of prooxidative processes in HDL-lipoprotein fraction were determined concentrations of malondialdehide (MDA), lipid hydroperoxide (LOOH), advanced oxidation protein products (AOPP), superoxide anion ($O_2^{\cdot-}$). The level of SH-groups was measured as parameter of antioxidative processes. We folowed total paraoxonase activity in serum.

Results: The levels of $O_2^{\cdot-}$, MDA, LOOH are significantly increased ($P< 0.001$), but level of SH-groups is significantly decreased ($P< 0.001$) in subgroups of pregnant women compared to control group. The highest changes are in second trimester. In group of sports players concentration of MDA, LOOH, AOPP, SH-groups are significantly increased ($P< 0.001$) compared to control group.

Conclusion: Our study has shown that pregnant women and professional sports players have increased levels of prooxidative parameters in HDL-lipoprotein fraction. Decreased concentration of SH groups in pregnant women and increased in sports players indicates to different antioxidative protection capability which is connected to changed paraoxonase activity.

Keywords: Oxidative stress, changed physiological states

Memory Stick

THE COMPARATIVE STUDY OF COMMON CAROTID INTIMA-MEDIA THICKNESS IN RUSSIAN AND GERMAN POPULATIONS

I. Sobenin[1,2], V. Myasoedova[1,2], T. Kirichenko[1,2], O. Chuprakova[2], M. Bauer[3], R. Erbel[3], **Alexander Orekhov**[2]

[1]Institute of General Pathology and Pathophysiology, [2]Institute for Atherosclerosis Research, Moscow, Russia, [3]University Duisburg-Essen, Essen, Germany

Background: Carotid intima-media thickness (cIMT) is a surrogate marker for generalized atherosclerosis and a risk factor for the development of atherosclerotic diseases. Increased cIMT has become a target for detecting subclinical atherosclerosis. However, the decision on normal and high values of cIMT is highly dependent of the difference between populations and the methodology used for its measurement.

Objective: We have compared the difference in cIMT distributions between population samples from Moscow (Russia) and Ruhr (Germany) regions.

Patients and methods: A subsample of 1740 participants of Heinz Nixdorf RECALL Study was compared to 1300 participants from Moscow, aged 45-75 and free from atherosclerotic diseases. High resolution ultrasonography of the distal centimeter of common carotid arteries was used for the measurement of cIMT, which was performed in the similar manner independently at both sites.

Results: A striking difference in cIMT distributions between population samples was revealed, which accounted for 0.182 mm (95% CI 0.162-0.202) in men, and 0.191 (95% CI 0.178-0.205) in women, $p< 0.001$. For men in Moscow, 25th and 75th percentile values of cIMT were 0.770 and 0.960 mm, as compared to 0.600 and 0.780 mm in Ruhr ($p< 0.001$). In women, these values accounted for 0.718 and 0.790 mm in Moscow, as compared to 0.543 and 0.696 mm in Ruhr ($p< 0.001$).

Conclusions: These findings demonstrate a geographical and socio-economic impact on the predisposition to atherosclerosis in different populations, as well as the role of cIMT for explanation of striking differences in cardiovascular morbidity and mortality between Russia and Germany.

Memory Stick

INFLUENCE OF PERIPHERAL ARTERIAL DISEASE ON LOWER EXTREMITY ARTERIAL STIFFNESS. THE CZECH POST-MONICA STUDY

Peter Wohlfahrt[1], D. Palous[2], M. Ingrischova[3], A. Krajcoviechova[1], J. Seidlerova[4], J. Bruthans[1], M. Galovcova[1], V. Adamkova[1], J. Filipovsky[4], R. Cifkova[1], The Czech post-MONICA Study Group

[1]Department of Preventive Cardiology, [2]Department of Radiology, Institute for Clinical and Experimental Medicine, [3]2nd Medical Department-Clinical Department of Cardiology and Angiology, 1st Medical Faculty, Charles University, Prague, [4]Center of Preventive Cardiology, 2nd Department of Internal Medicine, Charles University, Medical Faculty, Pilsen, Czech Republic

Increased lower extremity arterial stiffness leads to decreased blood flow in popliteal artery, decreases transcutaneous oxygen tension and is closely associated with symptoms of lower limb peripheral arterial disease. The aim of the present study was to access association between ankle-brachial index (ABI) as a parameter of lower extremity atherosclerosis and lower extremity pulse wave velocity (ePWV) as a parameter of arterial stiffness.

Methods: We included 884 patients from the Czech post-MONICA study (a randomly selected 1% representative population sample, mean age 54±13.5 years, 47% of men) with complete data on ABI and ePWV. ABI was measured using a handheld Doppler and ePWV using the validated Sphygmocor device.

Results: Surprisingly ePWV increased with increasing ABI ($r=0.15$, $p< 0.001$). Individuals with ABI below 0.9 had lower ePWV compared with subjects with normal (ABI 1-1.4) and high ABI (ABI>1.4) (8.1±1.9 vs. 9.8±1.8, $p< 0.05$; 8.1±1.9 vs. 10.6±1.3, $p< 0.005$). Individuals with ABI over 1.4 had significantly increased ePWV compared with low ABI (10.6±1.3 vs. 8.1±1.9, $p< 0.005$) and normal ABI groups (10.6±1.3 vs. 9.8±1.8, $p< 0.05$). These associations remained significant after adjustments for traditional cardiovascular risk factors.

Conclusion: Lower extremity pulse wave velocity in patients with peripheral arterial disease is artificially decreased probably due to blood pressure decrease and waveform changes behind significant stenosis. Subjects with incompressible arteries (ABI>1.4) have significantly increased ePWV.

Memory Stick

IS C-REACTIVE PROTEIN A BETTER PREDICTOR OF RECURRENT CAROTID DISEASE FOLLOWING CAROTID ENDARTERECTOMY THAN ESTABLISHED RISK FACTORS FOR ATHEROCLEROSIS?

Ivo Lusic[1], V. Radonić[2], S. Pavelin[1], I. Bilic[1]

[1]Neurology, [2]Vascular Surgery, University Hospital Centre and Medical Faculty, Split, Croatia

Background: To identify possible relation between established and novel risk factors for atherosclerosis (gender, age, diabetes mellitus, hypertension, smoking and C-reactive protein) and possibility of carotid restenosis following carotid endarterectomy (CEA).

Material and methods: A prospective study of 193 consecutive patients, admitted electively for carotid endarterectomy during 68 months, was conducted. 131 patients had symptomatic and 62 asymptomatic carotid disease. An attempt was made to follow-up on all operated arteries with duplex sonography at 2 month, 6 months, 12 months, and 24 months postoperatively. The correlation of previously mentioned variables (gender, age, diabetes mellitus, hypertension, smoking and average values of C-reactive protein) with progressive or recurrent disease was determined by chi-square analysis and analysis of variance.

Results: Of all 193 examined patients 29 demonstrated increasing degree of artery stenosis, while recurrent artery stenosis of >69% was diagnosed in 11 patients. Age, gender, hypertension, and diabetes did not play a significant role in the presence of progressive (or recurrent) disease, while active smokers and patients with preoperative and average C-reactive protein (CRP) levels over 3.0 mg/L had a greater propensity to develop progression (or recurrence) of carotid disease.

Conclusion: Increased levels of CRP in serum may be a better predictor of carotid restenosis after CEA than are cholesterol levels and other established risk factors for vascular disease - except active smoking.

Memory Stick

SINGLE DOSE PHARMACODYNAMICS AND PHARMACOKINETICS OF DRL-17822, A POTENT CETP INHIBITOR, IN HEALTHY SUBJECTS

Sumera Hasham, T. Mangapathiraju, S. Vittal, A. Bapat, M. Rao, S. Sai, A. Shanavas, K. Allenby, R. Kumar

Dr. Reddy's Laboratories Ltd., Hyderabad, India

Introduction: The inverse correlation between low HDL-C and cardiovascular risk has encouraged the development of cholesteryl ester transfer protein (CETP) inhibitors to raise HDL-C. DRL-17822 is an orally active potent small molecule CETP inhibitor. In preclinical models, DRL-17822 significantly increased HDL-C levels and was well tolerated with no observed effects on blood pressure or heart rate.

Objectives: DRL-17822 was evaluated for its pharmacodynamics and pharmacokinetics in a single ascending dose study in normal healthy subjects.

Methods: Seven groups of 6 subjects each were randomized to receive a single dose of either placebo or DRL-17822 (5, 25, 100, 300, 600, 800 and 1000 mg) under fasted state. Plasma CETP activity and concentrations of DRL-17822 were measured.

Results: DRL-17822 was well tolerated at all doses. DRL-17822 was gradually absorbed with maximum plasma concentrations being attained at 5 to 6 h post-dose; t_{max} was dose independent. There was a dose related increase in plasma exposure of DRL-17822 as well as plasma CETP inhibition from 25 to 600 mg. Plasma CETP inhibition became apparent as early as 2 hrs post dose and maximal inhibition was observed at 6 hrs. At t_{max} mean plasma CETP inhibition of 24, 32, 64, 92, 100, 100 and 96% compared to baseline was observed at 5, 25, 100, 300, 600, 800 and 1000 mg respectively. The CETP activity showed a trend towards returning to baseline activity by 24 h post dose.

Conclusions: DRL-17822 was well tolerated with favorable pharmacokinetics and dose dependent CETP inhibition in normal healthy subjects.

Memory Stick

IRBESARTAN REDUCES PLATELET ACTIVATION IN HYPERTENSIVE-HYPERCHOLESTEROLEMIC HAMSTER

Nicoleta Alexandru, A. Georgescu, D. Popov, E. Andrei, E. Dragan, M. Simionescu

Institute of Cellular Biology and Pathology 'Nicolae Simionescu', Bucharest, Romania

Altered platelet function and enhanced oxidative stress have been reported both in hypertension and in hypercholesterolemia. Among the proatherogenic agents, angiotensin II plays a considerable role in the development of atherosclerosis.

Aims: Assessment of the simultaneous effects of hypertension and hypercholesterolemia on platelet activation, oxidative stress, and nitric oxide (NO) production, and evaluation of the effect of irbesartan, an angiotensin II type 1 receptor antagonist employing an original hamster model.

Material and methods: The experiments were performed on platelets isolated from the blood of Golden Syrian hamsters divided in three experimental groups: controls, C, hypertensive-hypercholesterolemic, HH and hypertensive-hypercholesterolemic treated with irbesartan, HHI (fed as HH group plus irbesartan, 4 months).

Results: The new animal model, HH is characterized by:

(i) platelet activation as demonstrated by structural modifications, increased integrin 3 beta expression on plasma membrane and of proteins implicated in the activation and aggregation process (FAK, PI3K, Src);

(ii) a significantly higher endogenous generation of ROS in platelets, mostly produced by NADPH-oxidase;

(iii) enhanced expression of oxidase subunit gp91phox of NAD(P)H, 12-lipoxygenase, cyclooxygenase-1, $cPLA_2$, PKC protein expression, and

(iv) reduced platelet NO production (compared with C group).

Compared with HH group, the treatment with irbesartan (HHI) significantly attenuates the level of all the molecules tested.

Conclusions: Experimental hypertension associated with hypercholesterolemia induces major changes in blood platelets, which are significantly diminished by irbesartan administration. Moreover, this AT1-R antagonist reduces platelet ROS generation acting on the enzymatic sources involved in ROS production, suggesting an antioxidant effect of irbesartan.

Memory Stick

ONE-MONTH CONSUMPTION OF AN OLIVE LEAF EXTRACT ENHANCES CARDIOVASCULAR STATUS IN HYPERCHOLESTEROLEMIC SUBJECTS

Juristo Fonollá[1], P. Díaz-Ropero[1], E. de la Fuente[2], J.C. Quintela[2]

[1]R&D Department, Puleva Biotech, Granada, [2]R&D Department, Puleva Biotech, Madrid, Spain

Aims: To investigate the effects produced by a 28-day consumption of an olive leaf extract rich in polyphenols and triterpenic acids in cardiovascular and oxidant markers in healthy volunteers with high levels of total cholesterol. Security parameters also were studied.

Methods: It is a longitudinal, controlled, randomized, and double-blind intervention study. 39 subjects (45,0±8.8 years) of Granada (Spain) with more than 220 mg/dL of total cholesterol were randomly distributed into two study groups: the control group (CG, n=21, 44.4±9.6 years) that consumed placebo and the supplemented group (SG, n=18, 45.8±8.2 years) that consumed 1.200 mg/day of the olive leaf extract (Olivia®). Volunteers took four capsules at day: two after lunch and two after dinner. Blood samples were taken at 0, 14, 28 and 42 days (14 days after finished the intervention).

Results: The consumption of the olive extract induced significant decreases in total cholesterol (-7%), LDL-cholesterol (-12%), the total cholesterol/HDL-cholesterol ratio (8%), oxidized LDL (-13%), and gamma-GT (-28%) in the SG. LDL lag time increased significantly (9%). Plasma triglycerides, AST, ALT, creatinine and uric acid remained unchanged. All parameters return to original levels when volunteers stopped the consumption. There were no changes in the CG.

Conclusions: The after meal administration of an olive leaf extract improved cardiovascular risk markers and oxidative and hepatic parameters in hypercholesterolemic subjects.

Memory Stick

DAL-VESSEL - A RANDOMISED, PLACEBO-CONTROLLED STUDY OF DALCETRAPIB ON ENDOTHELIAL FUNCTION, AS MEASURED BY FLOW MEDIATED DILATATION, IN HIGH-RISK PATIENTS

Thomas Lüscher[1], S. Taddei[2], T. Münzel[3], J.W. Jukema[4], J. Kastelein[5], J.-C. Kaski[6], V. Lehnert[7], T. Burgess[8], D. Kallend[7], J. Deanfield[9]

[1]Cardiology, Cardiovascular Center, University Hospital Zurich, Zurich, Switzerland, [2]Hypertension Service, Università di Pisa, Pisa, Italy, [3]Department of Medicine II, Johannes Gutenberg-University Mainz, Mainz, Germany, [4]Department of Cardiology, Leiden University Medical Center, Leiden, [5]Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands, [6]Department of Cardiological Sciences, St George's, University of London, London, UK, [7]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [8]Hoffmann-La Roche Inc., Nutley, NJ, USA, [9]Cardiothoracic Unit, Great Ormond Street Hospital, London, UK

Background and aims: Dalcetrapib, a CETP inhibitor, effectively increases HDL-C and may reduce residual cardiovascular (CV) risk. Prolonged exposure to CV risk factors, including LDL-C, contributes to endothelial dysfunction, a precursor of atherosclerosis, while HDL-C appears protective. dal-VESSEL will investigate the effects of dalcetrapib, on top of standard care, on endothelial function, i.e. whether treatment will improve or unchange flow-mediated dilatation (FMD) of the brachial artery or even cause vasoconstriction. The effects of dalcetrapib on blood pressure (BP), and biomarkers of inflammation will also be assessed.

Patients and methods: Patients with CHD or CHD risk equivalents and HDL-C < 50 mg/dL, randomised to dalcetrapib 600mg po daily or placebo po daily will have endothelial function measured by FMD and 24-hour BP monitoring by ABPM. The protocolled time on study treatment is 36 weeks, and target sample size is 450 individuals. Co-primary endpoints are change from baseline in % FMD at 12 weeks and in mean BP at 4 weeks. Secondary endpoints include change from baseline in % FMD at 36 weeks and in mean BP at 12 and 36 weeks. Additional parameters include blood lipids, lipoproteins, biomarkers of inflammation, oxidation and CV risk, CETP mass and activity, adverse events, vital signs and ECG.

Results: To date 476 patients have been randomised in 19 centres in 6 countries.

Conclusion: dal-VESSEL is expected to increase our knowledge on the safety profile of dalcetrapib as part of the dal-HEART program, whilst providing insights into the use of FMD in a large multicentre setting.

Memory Stick

ANTI-ANGIOTENSIN DRUG EVALUATION IN APOE-/- MICE BY USPIO-ENHANCED MRI AT 7T

Monica Sigovan[1], A. Bessaad[1], E. Kaye[2], E. Lancelot[3], C. Corot[3], N. Provost[4], Z. Majd[4], M. Breisse[4], E. Canet-Soulas[1]

[1]CREATIS-LRMN, Lyon1 University, Lyon, France, [2]Radiology, Stanford University, Stanford, CA, USA, [3]Guerbet, Paris, [4]Genfit, Lille, France

Purpose: We investigated the effect of an angiotensin receptor blocker (Irbesartan) on the development of atherosclerosis.

Methods: The longitudinal study was performed non-invasively by USPIO enhanced MRI in apolipoprotein (apo) E-deficient mice fed a western-type diet at 10, 24 and 38 weeks of age. Baseline MRI was followed by the administration of a USPIO agent and by a second imaging session 48h post contrast. The surface of the ascending aorta was measured, and T2* maps were calculated.

Results: Vessel wall area increased significantly over time for both the non treated group ($p=0.001$) and the treated group (diet + Irbesartan) ($p=0.05$). Linear regression yielded a 1.8 times higher slope of vessel wall increase for the non treated group compared to the treated group. Significantly shorter mean T2* values were obtained at post-USPIO compared to baseline at every imaging point for both groups ($p< 0.001$) confirming the presence of USPIO, however no differences were found between groups.

Conclusions: Irbesartan lead to a marked reduction in the plaque formation. Concerning USPIO-enhanced MRI, the study failed to highlight differences between the two groups based on the post-contrast plaque T2* values. Literature data concerning renin-angiotensin system (RAS) blockers seems to indicate that responses depend on age, sex, dose, duration of treatment, and site of lesion investigation. Thus, a limitation of the current study is the fact that only the ascending aorta just above the sinus was evaluated by MRI, hence the obtained information may not be fully representative of the general effect of the drug.

Memory Stick

FACTORS INFLUENCING THE RISK OF INTENSIFICATION OF ANTI-HYPERGLYCEMIC MEDICATION AMONG ER NIACIN/LAROPIPRANT AND PLACEBO TREATED PATIENTS WITH TYPE 2 DIABETES

Alexandra Maclean[1], H. Giezek[2], C. Mccrary Sisk[1], D. Maccubbin[1]

[1]Merck Research Laboratories, Rahway, NJ, USA, [2]Merck Sharp & Dohme, Brussels, Belgium

Objective: Niacin improves diabetic dyslipidemia but also increases blood glucose levels in some patients. Compared to placebo (PBO), type 2 diabetes (DM-2) patients treated with ER niacin/laropiprant (ERN/LRPT) were shown previously to be more likely to require intensification of their anti-hyperglycemic medications (AHM; 8.2% vs. 17.6%; odds ratio and 95% CI: 2.4 [1.5, 3.8]). The present analysis was conducted to identify prognostic factors that influence risk of requiring AHM intensification.

Methods: This was a *post-hoc* analysis of a double-blind, PBO-controlled, 9-month study, in which DM-2 patients (n=796) were randomized 4:3 to ERN/LRPT (1g/d for 4 weeks and 2g/d thereafter) or PBO. The parameters were examined using univariate (Table) and multivariate logistic regression.

Results: Baseline HbA_{1c}, glucose, insulin use, and gender were strong predictors of AHM intensification regardless of treatment (Table). The types of AHM adjustments (dose increases, addition of new medications, etc.) were qualitatively similar for ERN/LRPT and PBO patients.

Conclusion: While more ERN/LRPT- versus PBO-treated DM-2 patients required AHM adjustments over 9 months, the predictors of risk of AHM adjustment as well as the overall types of adjustments required to manage glycemic status were similar in both treatment groups.

		Intensification of medication;			
Parameter	Group	Cases/total (%)	Odds Ratio	95% CI	P-value
Baseline HbA1c (%)	> median (6.8) < median(6.8)	82/387 (21.2) 25/398 (6.3)	4.01	(2.50, 6.43)	<0.001
Baseline glucose (mg/dL)	> median(129) < median(129)	78/388 (20.1) 29/397 (7.3)	3.19	(2.03, 5.02)	<0.001
Insulin user at baseline	Insulin user Non-user	31/154 (20.1) 76/635 (12.0)	1.85	(1.17, 2.94)	0.009
Gender	Female Male	55/311 (17.7) 52/478 (10.9)	1.76	(1.17, 2.65)	0.007

[Baseline Parameters]

Memory Stick

PHARMACOLOGIC INHIBITION OF HYALURONAN SYNTHESIS CAUSES LOSS OF ENDOTHELIAL GLYCOCALYX AND AGGRAVATES ATHEROSCLEROSIS

Nadine Nagy[1], T. Freudenberger[1], H. Jastrow[2], T. Suvorava[3], G. Kojda[3], A.A. Weber[3], S. Ergün[2], J.W. Fischer[1]

[1]Institut für Pharmakologie, [2]Institut für Anatomie, Universitätsklinikum Essen, Universität Duisburg-Essen, Essen, [3]Institut für Pharmakologie und Klinische Pharmakologie, Universitätsklinikum Düsseldorf, Düsseldorf, Germany

Background: Hyaluronan (HA) plays an important role in smooth muscle cell migration and proliferation, furthermore HA is thought to mediate neointimal expansion. However, hyaluronan also mediates vasoprotection as an integral component of the endothelial glycocalyx. The present study examines for the first time the effects of chronic pharmacologic inhibition of HA synthesis on atherosclerosis and vascular function.

Methods and results: ApoE-deficient mice on Western diet were treated orally with 4-methylumbelliferone (4-MU), an inhibitor of HA synthesis, beginning at 4 weeks of age. Subsequently, aortic atherosclerotic plaque burden and plaque size at the aortic root level were determined at 8, 15 and 25 weeks. Atherosclerosis was increased at 25 weeks and accumulation of mac2-positive macrophages was increased in the lesions. Furthermore, acetylcholine dependent relaxation of aortic rings was decreased and mean arterial blood pressure was increased in response to 4-MU. The model of photochemically induced thrombosis revealed a pro-thrombotic state that was not due to increased platelet activation as determined by CD62P expression. The pro-thrombotic state also did not reflect increased thrombin activation as monitored by the endogenous thrombin potential. Furthermore, the lesion promoting effect of 4-MU was specific for atherosclerosis since 4-MU did not accelerate neointimal hyperplasia in response to ligation of the left carotid artery. Of note, electron microscopy of myocardial capillaries revealed a severely damaged endothelial glycocalyx after 4-MU treatment.

Conclusion: The data suggest that systemic inhibition of HA synthesis accelerates atherosclerosis possibly due to ablation of the protective function of the endothelial glycocalyx.

Memory Stick

CHANGES OF SEMG ACTIVITY IN PROXIMAL AND DISTAL LEG MUSCLES IN PATIENTS WITH CLAUDICATION OVER 12-WEEK TREADMILL TRAINING-THE PILOT STUDY

Piotr Mika[1], A. Mika[1], L. Oleksy[2,3], A. Konik[1]

[1]Departament of Clinical Rehabilitation, University School of Physical Education, Krakow, [2]Zen Machines, Lancut, [3]The McKenzie Institute, Poznan, Poland

Introduction: Gait alteration is commonly occurring during walking in patients with claudication. A suggested mechanism underlying the abnormal gait in these patients is a weakness of the hip extensors and ankle plantar flexors.

Aim: To assess, for the first time, the changes of muscle bioelectrical activity in claudication patients over 12-week treadmill training.

Methods: Five patients with intermittent claudication were assigned into the 12-week treadmill-training program consisting of repeated walking bouts to onset of claudication pain. The surface electromyography (sEMG) of gastrocnemius lateralis, gastrocnemius medialis, tibialis anterior, biceps femoris, gluteus medius and rectus femoris muscles in claudicating leg were measured during treadmill testing (performed to maximal claudication pain) before and after 12- week training. The period between onset and offset of each muscle activity in the single gait cycle were evaluated. The values were normalized to Maximal Voluntary Contraction (MVC) and expressed as %MVC at the initial and at the terminal gait cycle of walking test.

Results: Pre-training bioelectrical activity of gastrocnemius lateralis was significantly ($p< 0.05$) higher at the terminal (133 % of MVC) than at the initial (50% of MVC) gait cycle of walking test. Such increase was not observed in post-training measurements ($p>0.05$) despite significant increase in walking distance. The post- to pre-training difference (98 % of MVC versus 199 % of MVC) in bioelectrical activity of gastrocnemius lateralis was also significant ($p< 0.05$).

Conclusion: Changes in calf muscles' bioelectrical activity may explain improvement of walking pattern in patients with claudication over 12- week treadmill training.

Memory Stick

COSTS AND CLINICAL OUTCOMES IN THE PATIENTS WITH OFF-PUMP VS. ON-PUMP CORONARY ARTERY BYPASS SURGERY

Tohid Emami Meybodi[1], S.K. Foruzannia[2], S.J. Mirhosseini[3], M.H. Abdollahi[3], H. Hosseini[3], S.H. Moshtaghion[3], A. Golzar[3]

[1]Cardiovascular Research Center, Yazd University of Medical Science, [2]Department of Cardiovascular Surgery, [3]Yazd University of Medical Science, Yazd, Iran

Introduction: The present era of the heath care places major emphasis on significantly reducing cost and resource utilization while maintaining quality of care and patient satisfaction. Off-pump coronary artery bypass surgery (OPCAB) avoids the potential complications of cardiopulmonary bypass, How ever; its acceptance depends on medical and economic out come. The aim of this study was to compare medical and economic out come of off pump and on pump surgery.

Methods: 304 patients undergoing first time coronary artery bypass surgery were randomized in to conventional on-pump and off pump group. Variables and fixed direct costs were obtained for each group and the data were analyzed using parametric methods.

Results: There was no difference between the groups with respect to pre- and intraoperative patient variables. OPCAB significantly reduced the incidence of postoperative transfusion requirement ($p< 0.05$) and showed a trend toward reduced morbidity which did not reach statistical significant ($p>0.05$). Although the use of an off pump procedure was associated with a decrease in risk adjusted operative mortality from 2.6% with conventional CABG to 0.7% in the off pump group but both off pump and on pump procedures produced excellent early clinical results. There were no statistically significant difference in surgical reexploration, length of stay and costs two groups.

Conclusion: Based on the results of this study there was no significant difference between OPCAB and on-pump CABG.

Keyword: On-pump CABG, Off-pump CABG, heart disease, cost

Memory Stick

ANTIPLATELET THERAPY IN PERIPHERAL ARTERIAL DISEASE: PRESCRIBING PRACTICE REMAINS SUB-OPTIMAL

Bilal Alkhaffaf, T. Breslin, S. Ahmad, M. Hadfield

Department of Vascular Surgery, Pennine Acute Hospitals NHS Trust, Oldham, UK

Objective: Peripheral arterial disease (PAD) is a greater predictor of myocardial infarction, stroke and death than coronary or cerebro-vascular disease. Despite this, PAD remains largely under-diagnosed and under-treated. Antiplatelet therapy (usually aspirin) is essential in the management of PAD, resulting in 22% fewer ischaemic events. Furthermore, the clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial suggests that Clopidogrel (Plavix) provides a 24% relative risk reduction of ischaemic events compared to aspirin. This effect is amplified in patients with multi-vascular bed disease. Our study aimed to evaluate antiplatelet use in PAD patients and identify factors influencing current practice.

Methods & patients: This was a prospective study of 200 consecutive patients with PAD.

Results: 122 patients (61%) were treated with aspirin, 20 (10%) used clopidogrel, while 39 (19.5%) received no anti-platelet therapy. The remaining 19 (9.5%) used appropriate alternative anti-platelets. 108 patients suffered from multi-vascular bed disease. In this group 64 (59%) were prescribed aspirin, 11 (10.2%) used clopidogrel, while 10 (14.8%) received no anti-platelet therapy. Prescription of clopidogrel for all suitable patients would require an 8-fold increase in spending.

Conclusion: One fifth of PAD patients receive no antiplatelet therapy. In addition, the use of clopidogrel, particularly those with multi-vascular bed disease, is extremely low which may be due to its high cost relative to aspirin. However the recent development of alternative generic clopidogrel should alleviate this problem.

Memory Stick

CYTOKINE PROFILES AND OXIDATIVE STRESS IN ACUTE CORONARY SYNDROME

Mahdi Sahlawi[1,2], Mohammed O. Hamza

[1]Biochemistry, College of Medicine/Kufa University, Kufa, [2]Biochemistry, College of Medicine/Nahrain University, Baghdad, Iraq

Background: Inflammation and oxidative stress play important role in atherosclerotic plaques rupture that leads to acute coronary syndrome (ACS).

Objective: This study was carried out to asses the cytokine profiles, inflammatory and oxidative status in ACS.

Patients and methods: A total of 125 individuals were enrolled in the study, 40 individuals were represented as healthy control group and the rest 85 subjects were patients presented to the emergency department ACS (myocardial infarction, MI or unstable angina pectoris, uAP). In addition to ECG, Creatine Kinase MB (CK-MB) enzyme, lipid profile, serum malondialdehyde (MDA) and Cytokines Profile; interleukine-1 beta (lL-1B) tumor necrosis factor alpha (TNFα) and interleukine-8 (lL-8) were measured.

Results: The serum levels of TNFα, lL-1B and lL-8 were significantly (P< 0.05) elevated in patients with ACS. Our data also showed a highly significant (P< 0.001) elevation in serum MDA in patients with acute MI and uAP. Positive correlation was observed between serum (MDA) and change in serum lipid profile in patients with ACS. Further positive correlation was noticed between IL-8 and CKMB in patients with Non Q-MI and uAP. In patients with ACS, a significant positive correlation was found between TG, TC and LDL and sialic acid (TSA and LSA and significant negative correlation between HDL-Cholesterol and TSA/ LSA.

Conclusion: Cytokine profile and oxidative stress markers were elevated in ACS. These markers could help in diagnosis of ACS and cardiovascular risk assessment.

Memory Stick

THE TWISTED CAROTID BIFURCATION AND MICROTHROMBENDARTERECTOMY

Hiroyuki Katano[1,2], K. Yamada[1]

[1]Neurosurgery, [2]Medical Informatics and Integrative Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Introduction: The twisted carotid bifurcation is a variant in which the internal carotid artery (ICA) courses medially to the external carotid artery (ECA). Since descriptions in the literature have been limited we have document clinical features and surgical experience of carotid endarterectomy (CEA) for cases of twisted carotid bifurcation.

Methods: Seventy-five out of a total of 106 consecutive cases with surgically treated carotid stenoses underwent CEA. Twisted carotid bifurcation was defined as a typically appearing ICA running medially in anterior-posterior view. The angle of twist was measured in the axial view of MDCT angiography as the medially deviated degree.

Results: Seven cases (9.3%) demonstrated twisted carotid bifurcations. Six found on the right side and one on the left. The average of angle of twist was 80.0 ± 17.6 degrees, while that with a normally positioned bifurcation was -7.4 ± 7.7 degrees. Six patients (85.7%) had diabetes mellitus and five patients (71.4%) had hypertension as a co-existing disease. Postoperative MDCT angiography revealed twisted position as in the preoperative state in four, complete correction to the normal position in two and half-corrected in one.

Conclusions: Twisted carotid bifurcations were preferentially found in right severely atherosclerotic carotids in patients with diabetes mellitus and/or hypertension. CEA of twisted carotids can be safely accomplished, sometimes with correction of the carotid position. MDCT angiography was useful for perioperative evaluation. Variation should be considered by the neurosurgeon and neurologists, concerned in the evaluation and treatment of carotid stenoses.

Memory Stick

ENDOTHELIAL DYSFUNCTION AND HMG-COA REDUCTASE INHIBITORS IN PATIENTS WITH ARTERIAL HYPERTENSION

Constantin Tudor[1], A. Iana[1], R.A. Jeberean[2], R. Buzas[1], A. Petrus[1]

[1]Internal Medicine, [2]Psihiarty, University Hospital Municipal Timisoara, Timisoara, Romania

Background and aims: The traditional cardiovascular risk factors as well as unknown risk factors contibuate to endothelial dysfunction and cardiovascular events. Endothelial dysfunction is a systemic disorder. The aims of the study were to lower the lipid profile and to improve the endothelial dysfunction in patients with arterial hypertension by the use of HMG-CoA reductase inhibitors.

Method and material: We enrolled 120 patients with a mean age 54.2 ± 7.8 years with arterial hypertension and without clinical changes of cardiovascular disease. Each patient received 40mg of HMG-CoA reductase inhibitors and antihypertensive treatment. The follow up period was of 5 years. In the first year every 3 months and afterwards every 6 months the patients underwent clinical exams, ECG, echocardiography and the endothelial dysfunction was measured by flow mediated dilatation (FMD) of the brachial artery on B-mode ultrasound images, with the use of a 7.0 MHz linear-array transducer in terms of fasting for 8 hours.

Results: After 5 years of follow up the characteristics of the studied group were: total cholesterol = 175.5 ± 15.5mg/dl, HDL-cholesterol = 48.2 ± 4.3mg/dl, LDL-cholesterol = 102.3 ± 8.5mg/dl, tryglicerides = 155.8 ± 16.2mg/dl.Table I FMD values before and after treatment with HMG-CoA reductase inhibitors : Parameters

FMD before treatment 7,3±2,5%

FMD after treatment 9,2±1,9%

p-ANOVA < 0,001

Conclusions: Patients with arterial hypertension had endothelial dysfunction present. Treatment with HMG-CoA reductase inhibitors improved the endothelial dysfunction in patients with arterial hypertension. No cardiovascular event appeared during the 5 years of follow up.

Memory Stick

TYPE 2 DIABETES: BLOOD PRESSURE CONTROL AND ENDOTHELIAL FUNTION ARE INFLUENCED BY NUTRITIONAL EDUCATIONAL PROGRAM

Maria Chiara Masoni, L. Ghiadoni, C. Consani, C. Scarpellini, S. Taddei, O. Giampietro, E. Matteucci

Internal Medicine, University of Pisa, Pisa, Italy

Background: Type 2 diabetic patients (T2DM) are characterized by endothelial dysfunction.

Aim: The aim of this study is to evaluate the effect of a nutritional and dietary counseling on BP control and endothelial dysfunction in T2DM.

Methods: Nineteen T2DM patients (age 61±5 years, 4 females) were provided of a by a 2-day 24 hour dietary recall at 1, 3, 6, 9 and 12 months. Blood pressure, endothelium-dependent and independent (sublingual glycerol trinitrate, GTN, 25 µg) vasodilation were assessed at baseline and after 12 months. Twenty age and gender matched healthy subjects were recruited as controls.

Results. At baseline T2DM had significantly ($p< 0.01$) lower FMD (4,3±1,9%) and FMD/GTN ratio (0.61±0.15) as compared to controls (6.9±2.1% and 0.91±0.22, respectively). Response to GTN was similar in T2DM (7.8±2.4%) and controls (7.6±3.0%). After 12 months, there was a significant ($p< 0.01$) reduction in systolic BP (from 145±17 to 133±8 mmHg) and a not significant reduction in diastolic BP (from 85±12 to 78±9 mmHg, $p=0.06$), HbA1c (from 7.7±17 to 7.1±0.8%, $p=0.18$) and plasma folate (from 6.1±8.4 to 11.4±8.5 mg/ml, $p< 0.001$).FMD (5.9±2.9%) and FMD/GTN ratio (0.84±0.61), but not response to GTN (7.4±1.7%) were also significantly ($p< 0.05$) improved. However, the improvement in FMD and FMD/GTN ratio was not related to changes in BP, metabolic control, plasma folate concentration or pharmacological treatment, but to sodium excretion reduction exclusively ($r=0,51$, $p< 0.005$).

Conclusions: A long term dietary counseling have positive effect on T2DM patients resulting in a significant better BP control and an improvement of endothelial dysfunction.

Memory Stick

WATER EXTRACTS OF CINNAMON AND CLOVE EXHIBITS POTENT INHIBITION OF PROTEIN GLYCATION AND ANTI-ATHEROSCLEROTIC ACTIVITY *IN VITRO,* AND *IN VIVO*

Seo-Ri Jin[1], K. Park[1], J.-H. Park[1], S.-H. Jung[2], K.-H. Cho[1], Aging Associated Vascular Disease Research Center

[1]School of Biotechnology, Yeungnam University, Gyeongsan, [2]Ottogi Corporation, Anyang, Republic of Korea

Advanced glycation end products contribute to the pathogenesis of diabetic complications and atherosclerosis. Water extracts of ground pepper, cinnamon, rosemary, ginger, and clove were tested for anti-atherosclerotic and anti-diabetic activity *in vitro* and *in vivo*. Cinnamon and clove extracts (at final 10 mg/mL) had the strongest anti-glycation activity (up to 88%), while rosemary and ginger extracts had 20% and 25% inhibition of nonenymatic glycation of protein, respectively. Extracts of clove, rosemary, and cinnamon (at final 10 mg/mL) had stronger ferric ion removal ability (FRA), while ginger and pepper had much weaker FRA ability. Cinnamon and clove had the strongest inhibition of activity against copper-mediated LDL oxidation and LDL phagocytosis by macrophages. Cinnamon or clove extracts had potent cholesteryl ester transfer protein (CETP) inhibitory activity in a concentration-dependent manner. Cinnamon or clove extracts exhibited hypolipidemic activity and anti-obesity activity in a hypercholesterolemic zebrafish model; the clove extract-treated group had a 68% and 80% decrease in serum cholesterol and TG levels, respectively. The clove extract-fed group had the smallest increase in body weight and height and the strongest antioxidant activity following a 5-week high cholesterol diet.

In conclusion, hydrophilic extract of cinnamon and clove showed potent activities to suppress the incidence of diabetes and atherosclerosis via strong antioxidant potential, prevention of apoA-I glycation and LDL-phagocytosis, inhibition of CETP, and hypolipidemic activity. These results suggest the potential to develop a new functional dietary agent to treat chronic metabolic diseases, such as hyperlipidemia and diabetes.

Memory Stick

EFFECT OF BICYCLE TRAINING ON BLOOD OXYGEN TRANSPORT INDICES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

Andrei Karol

Grodno State Medical University, Grodno, Belarus

The aim of the study was to investigate the effects of bicycle training (BT) on the blood oxygen transport indices (BOTI) in patients with acute myocardial infarction (AMI).

Methods: The study population included 43 patients with AMI and 20 healthy volunteers. Usual physical rehabilitation was carried out for group I (n=21) and BT for group II (n=22). The intensity of BT was 50% from the individual threshold load detected on the bicycle ergometer during 30 minutes for 14 days. The BOTI: pO2, pCO2, pH were measured using microgasoanalyzer. The hemoglobin-oxygen affinity was determined according to the p50 index (the blood pO2 corresponding to its 50% oxygen saturation).

Results: p50 values were diminished ($p < 0.05$), pO2 decreased ($p < 0.01$), pCO2 raised ($p < 0.01$), pH decreased ($p < 0.05$) compared to controls in both groups. The treatment in group I increased p50 on 4.0% ($p < 0.05$), other parameters didn´t change. BT in group II increased p50 on 13.7% ($p < 0.05$), increased pO2 ($p < 0.05$) and diminished pCO2 ($p < 0.05$). Maximal reached load (MRL) initially was 82.4±6.0W in group I and 80.0±4.0W in group II. MRL after treatment increased on 29% ($p < 0.05$) in group I and on 50% ($p < 0.01$) in group II. The number of metabolic units didn´t change in group I and increased on 22.1% ($p < 0.01$) in group II.

Conclusion: Our results indicate significant disturbances of BOTI in patients with AMI. BT corrects the BOTI, increases tolerance to physical activity in patients with AMI, what improves efficiency of the treatment.

Memory Stick

ST ELEVATED MYOCARDIAL INFARCTION AFTER INTRAVENOUS INJECTION OF HYOSCINE-N-BUTYLBROMIDE

Suleyman Kanyılmaz[1], C. Altın[1], T. Katırcıbaşı[2], A. Yıldırır[1], H. Muderrisoglu[1]

[1]Cardiology, Başkent University Hospital, Ankara, [2]Cardiology, Başkent University Hospital, Adana, Turkey

Introduction: Coronary vasospasm is one of the reasons for the acute coronary syndroms. Hyoscine-N-Butylbromide is an antispasmodic agent, widely used for painful spasms of the smooth muscles in GIS. Our case is the first case of acute myocardial infarction (AMI) probably related with Hyoscine-N-Butylbromide in the literature.

Case: A 49 years old man was consulted to cardiology department in the early hours of the morning because of chest pain. There is no known risk factors for coronary artery disease (CAD). On physical examination; his blood pressure was 120/75 mmHg and heart rate was 67 bpm. Cardiac oscultation and other systemic examination was normal. 12-lead electrocardiogram (ECG) revealed an ST elevation in the inferior leads and reciprocal ST depression in the lateral leads (Figure 1). The patient was diagnosed as acute inferior MI. He was transferred to catheterization laboratory for coronary angiography (CAG) in the first hour of the chest pain. CAG demonstrated normal coronary arteries (Figure 2-3). Blood chemistry tests revealed elevation of cardiac enzymes (CPK 696 IU/l [22-200], CK-MB 41 IU/l [0-25], and Troponin-I 3.4 ng/ml [0-0.06]). The ECG was normalised two hours after the chest pain (Figure 4). We learned that ´Hyoscine-N-Butylbromide' was administered intravenously before the patient ' s anginal symptoms. The patient was diagnosed as acute myocardial infarction type II and discharged on calcium channel blocker and ASA.

Conclusion: Some antispasmodic agents such as Hyoscine-N-Butylbromide may cause AMI by provacating tachycardia and the coronary vasospasm. Therefore in the emergency departments medication history should be evaluated carefully.

Memory Stick

ACHIEVEMENT OF THE TARGET GOAL FOR LDL-CHOLESTEROL BY ROSUVASTATIN IN JAPANESE HYPERCHOLESTEROLEMIC SUBJECTS

Saburo Nakano[1,2], G. Yoshino[2]

[1]Sagamihara Chuo Hospital, Kanagawa, [2]Division of Diabetes, Metabolism and Endocrinology, Toho University School of Medicine, Tokyo, Japan

Aim: The present study was conducted in order to clarify the effect

of rosuvastatin(RSV) on LDL-C lowering efficacy in Japanese

hypercholesterolemic subjects.

Methods: This retrospective observational study was conducted on 151

stain-naive and 65 statin-switch patients. They are treated with 2.5

mg daily of RSV for more than 12 weeks. Primary outcomes were changes in LDL-C levels and proportions of patients achieving their respective target goals for LDL-C of Guidelines for Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases for Japanese.

Results: In statin-naive patients, RSV significantly reduced LDL-C from 141.4 to 82.3 mg/dl ($p< 0.001$) and percent changes of acievement of target goal for LDL-C from 27.2 to 97.4 %. In primary prevention group, their respective percent achievement increased from 33.3, 45.2 and 20.6 % to 66.7, 100 and 96.5 %, respectively, while in the secondary prevention group, its percent achievement increased from 11.1 to 100% after treatment. Even in statin-switch patients, RAV significantly reduced LDL-C from 126.8 to 89.5 mg/dl ($p< 0.001$) and percent changes of achievement of target goal for LDL-C from 52.3 to 95.4 %. There was no significant adverse effect during this study.

Conclusion: RSV was effective in not only lowering LDL-C but also achieving the target goal for LDL-C of hypercholesterolemic subjects at every category of cardiovascular risk. Even in the patient group whose LDL-C levels were suboptimal on their statins, RSV significantly decreased their LDL-C levels and achieved the better target goals for LDL-C after stain switching without any side effect.

Memory Stick

VEGETABLE OILS IN THE PREVENTION OF OCULAR COMPLICATIONS RELATED TO ATHEROSCLEROSIS

Toihiri Said, O. Helal, H. Berrougui, T.-M. Jennifer, S. Cunnane, A. Khalili

Sherbrooke University, Sherbrooke, QC, Canada

Introduction: Retinopathy is one of the complications of cardiovascular diseases and is, with cataract, the leading causes of blindness among the elderly. It is a multifactor pathology that might be develop in the terminal phases of atherosclerosis. There is no or little effective treatment for this eye pathology. However, some components of vegetable oils are important in the structure and function of the retina. The purpose of this work is to explore biological properties of four vegetables oils in the prevention of retinopathy.

Methods: Four different vegetables oils were incubated with the pigment epithelial cells of the retina. The cytotoxicity of these vegetable oils was assessed by spectrofluorimetry using neutral red, yopro-1and H_2CFDA. Membrane fluidity was evaluated by the fluorescence anisotropy with DPH. Inflammation was evaluated by the measurement of ICAM protein expression by flow cytometry and membrane fatty acid composition was assessed by gas chromatography.

Results: None of the tested oils is necrotic, apoptotic, or pro-inflammatory. In vitro, oil rich in zeaxanthin and lutein present no beneficial effect on the biophysical properties of the membranes. However, omega-3 rich oil improves the membrane fluidity by 48% compared to control. Moreover incubation of retina cells with omega-3 rich oil increases linolenic acid membrane composition by 230% after 72 hours incubation.

Conclusion: Our results show that vegetable oil components can incorporate in retina cells without inducing cytotoxicity. These results support clinical studies showing beneficial effects of omega-3 in retina function.

Memory Stick

EFFECT OF ROSUVASTATIN ALONE AND COMBINATION OF HMC05 AND ROSUVASTATIN ON ATHEROSCLEROTIC C57BL/6 APOE KNOCKOUT MICE

Joo Yun Kim[1,2], B.-S. Lee[1], H.-M.S. Shin[2], J.E. Park[1]

[1]Division of Cardiology, Samsung Medical Center & Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, [2]Department of Physiology, Dongguk University College of Oriental Medicine, Sukjangdong, Kyongju, Republic of Korea

Background: Atherosclerosis is understood as a chronic inflammatory disease of arteries. Previously, we have shown that HMC05, an herbal extract has anti-inflammatory and anti-atherosclerotic effects in ApoE-/- mice. In the present study, we have investigated the significance of the differences of the anti-atherosclerotic activities between single and concurrent administration of HMC05 and Rosuvastatin, a cholesterol lowing drug in ApoE-/- mice.

Method: ApoE-/- mice were fed with a high-fat high-cholesterol diet to induce atherosclerosis. Lipid accumulation in aortic arch and aortic sinus were measured by oil red O staining. Macrophage infiltration was examined by immunohistochemistry.

Result: When HMC05 (25mg/kg~100mg/kg) and Rosuvastatin (20mg/kg) were administered orally alone or in combination, both of the concurrent and single administration inhibited significantly the swelling of atherosclerotic plaque in ApoE-/- mice. Whereas there were not any significant differences between the HMC05 single administration and combined administration. In macrophage infiltration, the concurrent administration of HMC05 and Rosuvastatin showed non-synergistically effects than HMC05 single administration.

Conclusion: The result suggest that the anti- atherosclerotic effects are intensified by the concurrent administration of HMC05 and Rosuvastatin, but the anti- atherosclerotic effects of enough dose of HMC05 (25mg/kg) is comparable to that of the combination preparation.

Memory Stick

ENGINEERED BEE HYALURONIDASE AS A POTENTIAL TOOL FOR THE TREATMENT OF ATHEROSCLEROSIS

Attila Iliás[1], B. Greiderer-Kleinlercher[1], S. Reitinger[2], G. Lepperdinger[1]

[1]*Extracellular Matrix Research, Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria,*
[2]*Department of Chemistry, University of British Columbia, Vancouver, BC, Canada*

Atherosclerotic cardiovascular disease is the major cause of death in Western countries and its incidence is rapidly growing. Atherosclerosis is a chronic inflammatory disease. Atheromatous lesions, evolved in the arterial tunica intima are made of excess fat, collagen, elastin and hyaluronan. Hyaluronidases are expressed in prokaryotes and eukaryotes. Hyaluronidase from honey bee (*Apis mellifera*) shares 30% of sequence identity with human hyaluronidases, which are involved in fertilization and the turnover of hyaluronan. In contrast to human hyaluronidases, having pH optima at acidic pH, bee hyaluronidase exhibits activity at neutral pH as well. Recently, bee venom hyaluronidase has been produced in large quantity in *Pichia pastoris,* and its biochemical characterization has been performed.

The aim of our study is to utilize engineered hyaluronidase to remove hyaluronan from the developing lesions within arteries and in this way, to prevent further progression of atherosclerosis.

Wild-type bee hyaluronidase and engineered form of bee hyaluronidase, targeted to developing lesions were constructed and produced in large quantity in *Pichia pastoris*. To study the transport of the recombinant enzymes through activated endothelium an *in vitro* model was established. Our results show that the targeted version of bee hyaluronidase can bind to the activated endothelium and both enzymes can be transported through bEnd.3 cell monolayer. Our hypothesis is tested *in vivo*, using ApoE-deficient mice maintained on Western-type diet, administering the recombinant enzymes by tail vein injection.

Memory Stick

COMPARATIVE STUDY OF CAROTID PLAQUES OF PATIENTS AFTER ENDARTERECTOMY PROCEDURE

Svetlana V. Kashirina, V.S. Shishkina, E.R. Andreeva, O.P. Ilyinskaya, N.B. Radukhina, V.V. Ahmetov, E.M. Tararak

Cardiocenter, Moscow, Russia

Background: To find out correlation between morphometric parameters of endarterectomy plaques and clinical data of patients.

Methods: Thirty-five patients (15 symptomatic persons after stroke and/or transitory ischaemic attack, mean age 62.4 ± 10.9 years (group I), and 20 asymptomatic 64.3+7.4 years (group II), admitted for carotid endarterectomy were enrolled. A morphometric analysis was performed to characterize different parts of endarterectomy plaques and calculate percent of plaque square occupied with “hard” (caps and organized core connective tissue, calcium deposits) and “soft” (inflammatory cells accumulation, atheronecrotic core, vascularisation area). “Hard” /“soft” ratio was used to describe morphometric data. Histological and clinical data of groups I and II patients were compared using t-test analysis.

Results: 98% plaques of group I and only 65% of group II were typeVI (according AHA classification). Almost all group I plaques and 55% of group II had features of vulnerability. Only group I plaques had surface defects and surface thrombus. Two groups of patients significantly differed in plaque vascularization and vulnerability coefficient (Lovett et al, 2005), percent of stenosis, blood velocity and echogenicity. Marked differences between groups were find out in “hard” /“soft” ratio, calcium deposits and foam cells in plaque caps, cores and shoulders, plaque core associated hemorrhages and thrombus, diabetes mellitus, arterial hypertension.

Conclusion: Group I patients in compare with group II often had vulnerable atherosclerotic lesions with high risk of rupture. Asymptomatic carotid stenosis should be followed close with no invasive diagnostic methods and clinical evaluation.

Funding: RFBR-OFI-c-08-04-13753, contract N/10-ATH-M.

Memory Stick

ALUMINIUM PHTALOCYANINE ACCUMULATION IN ENDARTERECTOMY CAROTID PLAQUES: STUDY IN VITRO

Yulia Efremova, G.N. Soboleva, E.R. Andreeva, N.B. Radukhina, E.M. Tararak, Y.A. Karpov

Cardiocenter, Moscow, Russia

Background: Photodynamic approach is assumed as a perspective method tor atherosclerotic lesions detection in human arteries. Accumulation of photosensitizer in vascular wall is a critical point in this procedure. The aim of this study was to reveal phtalocyanine uptake in endarterectomy carotid plaques after in vitro loading.

Methods: Thirty plaques after carotid endarterectomy were cultured overnight in DMEM with 10% FCS and 10 ug/ml of aluminium phtalocyanine (Al-PS, NIOPIK, Russia), carfully washed out and cryostate sections were prepared. Sections were analysed in fluorescent microscope Leika DM 5000B and morphometric analysis was performed to reveal Al-PS accumulation in different parts plaques. Areas differed in Al-PS uptake were characterized with histologic and immunocytochemical approaches.

Results: 2-3 pecies of each plaque were analysed. Al-PS preferentially accumulated in plaque's caps, shoulders, connective tissue organized plaque cores and vascularization areas. These areas had highest fluorescent intensity and were characterized with high cellularity and immunocytochemically these cells were identified as macrophages, lymphocytes and smooth muscle cells.

Conclusion: Al-PS accumulation was assosiated with cellular components in endarterectomy plaques and may be a useful tool for imaging of metabolically active areas of atherosclerotic lesions.

Findings: Contract №8/3-408н-09.

Memory Stick

EFFECTS OF ATORVASTATIN ON POSTPRANDIAL TRIGLYCERIDE LEVELS AND CAROTID INTIMA-MEDIA THICKNESS IN NONDIABETIC DYSLIPIDEMIC PATIENTS

S. Isik[1], **Nafiye Helvaci**[2], K. Helvaci[2], U. Ozuguz[1], Y. Ates Tutuncu[1], D. Berker[1], S. Guler[1]

[1]Endocrinology and Metabolism, [2]Internal Medicine, Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey

Objective: Hypertriglyceridemia is an important risk factor for coronary heart disease. Accumulating evidence suggests that postprandial triglycerid (pTG) levels may be more closely related to the atherogenic risk than fasting triglyceride levels. To investigate the role of pTG in atherosclerosis in nondiabetic individuals and effect of atorvastatin, we examined pTG levels and carotid artery intima-media thickness (IMT) before and after treatment.

Methods: Carotid artery IMT was determined by high resolution B-mode ultrasonography in 30 newly detected hyperlipidemic, nondiabetic subjects [mean age was 43.3±5.7 (29-51), 22 female and 8 male] and 20 healthy control subjects. Plasma lipid parameters were measured after overnight fasting and TG 4. hour after standart meal (a total energy of 9 kcal/kg, including 60-65% carbohydrate, 15-20% protein, and 20% by fat). Patients were treated with atorvastatin (20 mg daily) for 6 months. After the treatment period, laboratory tests and CIMT measurements were repeated.

Results: Carotid IMT of the dyslipidemic patients was greater than the normolipemic subjects (0.77 ± 0.12 vs. 0.65 ± 0.13 mm; p=0.05). Carotid IMT measurements and LDL-cholesterol levels were significantly decreased from baseline levels (0.77±0.12 to 0.67±0.09, p< 0.001, and 185.9±21.8 to 107.2±19.5, p< 0.001). Significant improvement of TG levels (mg/dl) was observed after atorvastatin treatment (0.hour: 161.0±63.3, 97.1±22.7; 2.hour: 212.4±77.5, 123.2±32.3; 4.hour: 251.3±89.1, 149.4±37.3, respectively) (p< 0.001). A significant correlation did not observed between the decrease of CIMT with the changes in the lipid parameters levels after treatment.

Conclusions: Atorvastatin reduces CIMT regardless of lipid parameters in nondiabetic dyslipidemic patients.

Memory Stick

ARTROFOON AS THE NOVEL IMMUNE MODULATOR FOR IMPROVEMENT RESULTS OF TREATMENT IN PAD PATIENTS AFTER LATE THROMBOSIS

Alexei Svetlikov[1,2]

[1]St-Petersburg State Medical Academy, [2]Hospital 122, Saint Petersburg, Russia

The role low doses anti-TNF in improvement of immune function in PAD patients after late thrombosis.

Aim: To study the influence of low doses anti-TNF the function of immune system in patients with late thrombosis in case peripheral vascular surgery.

Materials/methods: The first group comprised 191 patients with late thrombosis of infrainguinal segments arteries taken the new developed drug artrofoon (contained low doses of anti-TNF antibodies) postoperatively for 2 months. The second group included similar 184 patients without using artrofoon. The third group, control group consisted of 95 patients without clinical and electrophysiological signs of atherosclerosis.

Results: The comparative analysis indicated, that patients of the first group had the statistically significant decrease of IgG (16,7+1,4g/l) and circulated antigen-antibody complexes (128+1,1), CD3 (till 0,74+0,03*109) , CD4 (0,46+0,04*109), IL-1, IL-6. The immune homeostasis disorders were accompanied not only with suppression but also with autoagression and inflammation development. The immunological procedures showed in the first group improvement of phagocytic number of neutrophiles, monocytes and their phagocytic index.

Conclusion: The postoperative treatment of PAD patients with late reocclusion may include low doses of anti-TNF antibodies. The final conclusion, recommendation to take this type of medicine in patients with severe atherosclerosis requires the further investigations.

Memory Stick

THE EFFECT OF ROSUVASTATIN IN THE ARTERIAL PRESSURE OF HYPERLIPIDAEMIC AND HYPERTENSIVE PATIENTS

Alexandra Sianni[1], A. Ganotopoulou[2], A. Kosma[3], E. Kakava[4], K. Liatsos[5]

[1]General Hospital of Athens Constantopouleio, Athens, [2]Pathology, General Hospital Tzaneio, Piraeus, [3]Psychiatric Hospital of Athens Dromokaiteio, [4]Private Pathologist, [5]General Hospital Mitera, Athens, Greece

Introduction: The3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (statin) inhibitors are extensively used to treat hypercholesterolaemia mainly because they are effective in reducing high levels of LDL-cholesterol.

Objective: To study the action of a representative of statins, i.e. of Rosuvastatin, in the arterial pressure of patients with LDL-cholesterol >130mg/dl.

Material and method: The study included 178 subjects, 68 men (38%) and 110 women (62%), having diagnosed arterial hypertension (AH) under medical treatment, and hyperlipidaemia with LDL-cholesterol >130mg/dl. The patients were split randomly into 2 groups. Group A included 78 patients with A Hunder treatment and hyperlipidaemia against which a hypolipidaemic diet scheme was administered for a period of 12 weeks. Group B included 100 patients with A Hunder medical treatment and hyperlipidaemia against which 20mg Rosuvastatin for 12 weeks was administered. During the 3-month follow-up the patients´arterial pressure (Systolic Arterial Pressure, SAP and Diastolic Arterial Pressure, DAP) was being recorded in the doctor's office twice weekly.

Results: A statistically non-significant decrease in the AP levels with a mean value of SAP-2.2mmHg andDAP-1.6mmHg was observed in the 78 subjects of Group Aconsisting of 22(28%) men and 56(72%) women. A statistically significant decrease in the arterial pressure levels with a mean value of SAP-5.8mmHg and DAP-2.6mmHg was observed in the 100 subjects of Group Bconsisting of 46(46%) men and 54(54%) women.

Conclusion: Among the pleiotropic actions of statins and particularly of 20mg Rosuvastatin, it appears that this statin has a beneficial effect on reducing the arterial pressure of hyperlipidaemic-hypertensive patients. Mechanisms that could explain the effect of statins on A Preduction are the following:

a. The arterial elasticity is improved and the atherosclerotic disease process is delayed through the inhibition of the vascular smooth muscle cell proliferation, which can lead to a long-term drop in AP.
b. The production of NO from the vascular endothelium is increased and the endothelial function is improved, which results in increasing arterial vasodilation that is directly related to the drop of AP.
c. Effect in the renin-angiotensin system through decrease in angiotensinIIreceptor type1, decrease in aldosterone levels in plasma without influencing the action of renin, and increase in urinary Naexcretion.

Memory Stick

BENEFICIAL EFFECT OFROSUVASTATIN ON THE ARTERIAL PRESSURE REDUCTION OF HYPERLIPIDAEMIC AND HYPERTENSIVE PATIENTS WHO RECEIVE DIFFERENT CATEGORIES OF ANTI-HYPERTENSIVE TREATMENT

A. Sianni[1], **Asimina Ganotopoulou**[2], A. Kosma[3], E. Kakava[4], K. Liatsos[5]

[1]General Hospital of Athens Constantopouleio, Athens, [2]General Hospital Tzaneio, Piraeus, [3]Psychiatric Hospital of Athens Dromokaiteio, [4]Private Pathology Practice, [5]General Hospital Mitera, Athens, Greece

Objective: To study the effect of 20mg rosuvastatin on the arterial pressure of hyperlipidaemic patients with diagnosed arterial hypertension who receive different categories of antihypertensive treatment.

Material and method: The study included 100 hyperlipidaemic patients, 46(46%) men and 54 (54%) women, with diagnosed arterial hypertension. To treat hyperlipidemia all patients have been receiving 20mg rosuvastatin for a period of 12 weeks. To treat arterial hypertension all patients received different categories of antihypertensive treatment; in particular, 42 patients received angiotensin T2 antagonist, 28 patients received a-MEA, 23 received diuretics and 7 patients received other anti-hypertensive treatment.

Results: Anti-hypertensive treatment /Number of patients/Mean change of Arterial Pressure as compared to baseline levels before the administration of 20mg rosuvastatin [SAP(mmHg)/DAP (mmHg)], Angiotensin T2 antagonist 42 (42%) 6.4 3.4, α-MEA 28 (28%) 5.8 3.1, Diuretics 23 (23%) 4.7 2.6, Other 7 (7%) 3.8 2.1.

Conclusion: Administering 20 mg rosuvastatin and anti-hypertensive treatment can improve hyperlipidemia and can substantially contribute to the reduction of arterial pressure in hyperlipidaemic and hypertensive patients. It is observed that a combined administration of rosuvastatin and angiotensin T2 antagonist can lead to a more significant decrease in SAP and DAP as compared to the co-administration of other types of anti-hypertensive medicines.

Memory Stick

AN IMPACT OF DRUG THERAPY ON CORONARY ARTERY DISEASE COURSE IN PATIENTS UNDERWENT MYOCARDIAL REVASCULARIZATION

Alexey Panov, K. Korzhenevskaya, V. Kozulin, M. Gordeev, N. Tayn

Federal Almasov Heart Blood and Endocrinology Center, Saint-Petersburg, Russia

Objective: To evaluate a postoperative drug therapy effect on clinical coronary artery disease course in patients underwent myocardial revascularization.

Methods: We observed 130 patients aged from 33 to 79, who underwent coronary artery bypass grafting (CABG) at the moment of non-ST elevation acute coronary syndrome (non-ST ACS). Most patients were men. There was multiple coronary arterial involment in most cases. The time of follow-up was 4 years. The typical anginal syndrome confirmed by stress-echocardiography test served as a recurrent angina assessment criteria. All patients after surgery were treated with antiaggregants, beta-adrenoblockers, statins, angiotensin converting enzyme inhibitors.

Results: None of patients had died within the follow-up. Recurrent myocardial ischemia was developed in the 35 patients. The analysis of compliance to drug therapy revealed that the majority of patients took antiplatelet agents (95.1 %) and statins (94.6%) in the postoperative period, only 6 patients withdrew them without assistance; 40 patients didn't take ACE inhibitor because of intolerance, 10 of them took angiotensin receptor blockers; and 5 patients withdrew beta-adrenoblockers without assistance and 10 patients took calcium-blockers. The patients with no angina and the patients with recurrent myocardial ischemia after CABG had no significant difference in postoperative drug administration frequency except antiaggregants. The recurrence rate was significantly higher in the patients discontinued antiplatelet therapy than in patients continuing to take antiaggregants postCABG (83.3% and 24.2% respectively, $p< 0.01$).

Conclusions: Antiplatelet agent therapy withdrawl after myocardial revascularization is associated with high reccurent angina rate.

Memory Stick

SAFETY AND TOLERABILITY OF ATORVASTATIN: ANALYSIS OF 8755 PATIENTS IN 17 PLACEBO-CONTROLLED TRIALS

Rachel Laskey, L. Tarasenko, R. Fayyad

Pfizer Inc., New York, NY, USA

Objective: To provide an updated, comprehensive assessment of the safety and tolerability of atorvastatin across the approved dose range.

Methods: Pooled data were analyzed from 16,066 patients (aged 10-94 years; 62% male) in 17 placebo-controlled trials (cut-off date 3/20/2008). The studies included 8755 patients on atorvastatin (3908 on 10 mg; 188 on 20 mg; 604 on 40 mg; 4055 on 80 mg) and 7311 on placebo. Median treatment duration was 53 weeks.

Results: Overall, incidence of treatment-associated adverse events (AEs) was similar between patients receiving atorvastatin (22.3%) and those receiving placebo (20.2%), and infrequently resulted in study withdrawal (atorvastatin = 5.2%; placebo = 4.0%). The most frequent treatment-associated AEs were gastrointestinal disorders (atorvastatin = 8.5%; placebo = 7.3%). Treatment-related muscle AEs were uncommon; myalgia rates were 2.1% for atorvastatin and 1.8% for placebo, and myopathy rates were ‹0.1% in both treatment groups. Rates of rhabdomyolysis were 0.02% for atorvastatin and 0.03% for placebo. Overall incidence of AEs was similar across the atorvastatin dose range, and serious nonfatal AEs were rare (atorvastatin ‹1%; placebo = 2.0%). In total, 37 patients (0.4%) receiving atorvastatin reported persistent elevations in ALT, AST, or both ≥3×ULN; 13 patients (0.2%) receiving placebo had persistent ALT elevations ≥3×ULN (8 of these also had persistent AST elevations). Two patients (‹0.1%) receiving atorvastatin (both at the 80 mg dose), had persistent CPK elevations ≥10×ULN.

Conclusion: Atorvastatin, across the 10- to 80-mg dose range, is generally safe and well-tolerated in a broad spectrum of dyslipidemic patients with varying cardiovascular risk.

Memory Stick

CORONARY CIRCULATION AND LEFT VENTRICULAR FUNCTION: EFFECT OF REVASULARIZATION IN PATIENTS WITH PRESERVED LEFT VENTRICLE SYSTOLIC FUNCTION

Olexander Kuryata, Y. Yegorova

Department of Hospital Therapy #1, Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine

Coronary artery disease (CAD) is one of the most frequent etiologies of chronic heart failure (CHF). The role of revascularization in treatment and prevention CHF in patients with CAD and preserved left ventricle systolic function is discussing. Aim. To asses the interrelation between coronary circulation and left ventricular contractile function, and influence of revascularization on CHD progression in patients with CAD and preserved left ventricular ejection fraction (LVEF). Material and methods. 78 pts with CAD were divided into two groups: 1^{st} group - 38 (48.71%) underwent coronary revascularization, 2^{nd} group - 40(51.28%) received only conservative treatment. One-vessel disease has 33(42.31%) pts, two-vessels - 28(35.90%) and multi-vessels disease - 17(21.79%) pts. Criteria excluding was LVEF< 40%. Left ventricular dimensions, 6-minute walk test were measured on baseline and after 1-year treatment. Results. In patients with multi-vessels disease the end-diastolic dimension was increased (P< 0.05). The NYHA class of heart failure improved in 1^{st} group (2.42±0.21 vs 1.80±0.17, P< 0.001) and in 2^{nd} group (2.39±0.24 vs 1.92±0.18, P< 0.01), but in 1^{st} group significantly higher than in 2^{nd} group. In 2^{nd} group in 5 pts class was increased. The left ventricular diastolic dimension decreased on 20.4% in 1^{st} group comparable 15.8% in 2^{nd} group (P< 0.05). LVEF significantly improved on 16.4% in 1^{st} group comparable 12.7% in 2^{nd} group (P< 0.05). 6-minute walk test was significally higher in 1st group (P< 0.05). Conclusion. Patients with multi-vessels disease had increased end-diastolic dimension. Revascularization had positive effect on LV contractile function and CHD progression.

Memory Stick

TOCOTRIENOL-RICH FRACTION SUPPLEMENTATION BEFORE CHOLESTEROL FEEDING PROVIDES PROTECTION AGAINST EARLY ATHEROSCLEROSIS

Azlina A Razak, E. Omar, N.A. Muhammad, N.H. Mahamad Rodi, H. Nawawi

Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Malaysia

Background: Tocotrienols have antioxidant properties. Oxidative stress plays an important role in atherogenesis. The role of tocotrienol-rich fraction (TRF) as a protective agent against early atherosclerosis is uninvestigated.

Objective: To determine protective effect of TRF supplementation in rabbits with early atherosclerosis.

Methods: 23 New Zealand white rabbits were randomly divided into two groups: A and B. Group A (N=13) had 60 days of normal diet (ND) followed by 14 days high cholesterol diet (HCD). This group was divided into two: TRF 15mg/kg and placebo, which was given throughout the study. Group B (N=10) had 14 days of HCD followed by 60 days ND. The group was also divided into two: TRF 15mg/kg and placebo, given after the cessation of HCD and continued until the end of the study. Fasting serum lipids were taken at baseline, 2, 8 and 10 weeks. At the end of the study, the rabbits were euthanized and their aorta evaluated for atherosclerotic lesion using Sudan IV stain. Quantitative analysis of the lesion was performed using image analysis software.

Results: A rise in serum lipids were observed in all groups after HCD, but no differences between the intervention groups was demonstrated. In group A, significantly less atherosclerotic lesion was seen in TRF treated group compared to placebo (7.9 ± 2.9 % vs. 16.6 ± 1.6 % respectively, $p< 0.05$). However, the lesions in group B were not significantly different.

Conclusion: TRF supplementation before cholesterol feeding reduces the formation of early atherosclerosis in rabbits.

Memory Stick

FDG-PET/CT AND MRI EVALUATION OF THE EFFECT OF DALCETRAPIB ON ATHEROSCLEROTIC PLAQUE PROGRESSION/REGRESSION: DESIGN OF THE DAL-PLAQUE STUDY

Zahi Fayad[1], M. Farkouh[1], A. Tawakol[2], J. Rudd[3], M. Woodward[1], L. Yang[1], J. Bucerius[1], C. Calcagno[1], T. Burgess[4], J. Pozza[4], V. Mani[1], D. Kallend[5]

[1]Mount Sinai Medical Center, New York, NY, [2]Massachusetts General Hospital, Boston, MA, USA, [3]Addenbrooke's Hospital, Cambridge, UK, [4]Hoffmann-La Roche Inc., Nutley, NJ, USA, [5]F. Hoffmann-La Roche Ltd, Basel, Switzerland

Background: Many patients remain at high cardiovascular (CV) risk despite optimal standards of care, including treatment with statins. Population studies have shown an inverse relationship between HDL-C concentration and CV risk. CETP inhibitors increase HDL-C levels, thereby potentially reducing CV risk and atherosclerotic burden.

Aims: To assess the effect of dalcetrapib, a selective inhibitor of CETP, on atherosclerosis using MRI and FDG-PET/CT.

Methods: dal-PLAQUE is a phase IIB, randomised, double-blind, placebo-controlled study in male and female patients (18-75 years) with CHD, or CHD risk equivalents, treated with LDL-C lowering drugs to a stable acceptable LDL-C level (< 100 mg/dL, < 2.6 mmol/L). The effect of dalcetrapib on change from baseline in target plaque to background blood ratio from an index vessel (right carotid, left carotid or ascending aorta) using FDG uptake measured with PET/CT after 3 and 6 months (latter-primary endpoint) and change from baseline in MRI plaque size/burden (wall area, wall thickness, and total vessel area) from an index vessel (right carotid, left carotid or thoracic aorta) after 6, 12 (co-primary endpoint) and 24 months will be evaluated. Changes in aortic compliance and dynamic contrast (gadolinium) enhanced MRI-derived parameters of extent of plaque macrophages and neovasculature will be evaluated after 6, 12 and 24 months. Additionally, impact of dalcetrapib on lipids, lipoproteins and serum inflammatory markers will be assessed.

Conclusion: Findings from dal-PLAQUE may provide important insights into the effect of CETP inhibition on atherosclerosis as measured by FDG-PET and MRI in patients with CHD or CHD risk equivalents.

Memory Stick

SAFETY CONSIDERATIONS IN INTERNAL CAROTID ARTERY STENTING

K. Papadopoulos[1], A. Tsanis[1], **Spiridon Volteas**[2], N. Batakis[1]

[1]Red Cross Hospital, [2]Hippokration Hospital, Athínai, Greece

The aim was to identify safety of ICA stenting using embolic protection (EPD) in patients with >80% ICA stenosis.

250 patients with symptomatic, >80% ICA stenosis were allocated into three groups. In Group A (168 patients with unilateral ICA stenosis), the balloon EPD was used in 127 and the Emboshield filter in 41. In Group B (31 patients with ipsilateral ICA stenosis with total occlusion of the contralateral ICA), the balloon EPD as used, as the method of choice but, when put in place with the balloon fully inflated, 22 patients could not tolerate cerebellar ischemia and direct stenting (unprotected) was applied. In Group C 51 patients (102 procedures with bilateral stenosis), underwent two-step stenting, with the opposite ICA stented 3-5 days later. The balloon EPD was used in 38 patients (76 procedures) and the Emboshield filter in 13 (26 procedures). Overall, in 281 procedures (168+31+102), EPD´s were successfully placed in all but finally used in 259 cases (92.17%).

All patients had negative post-procedure neurological examination. No death or major complication occurred. No TIA's were seen. One patient reported an ipsilateral arm weakness, but CT scan was negative. Only 5 minor complications occurred, one reperfusion syndrome (Group C) and 4 small haematomas at the puncture site. On Color Doppler, performed one-year later no severe restenosis (>50%) was seen.

In conclusion, EPD placement was feasible all cases making ICA stenting using embolic protection a safe and effective intervention. These short and intermediate results are fully comparative (if not better) to endarterectomy.

Memory Stick

PITAVASTATIN 4MG SHOWS COMPARABLE LDL-CHOLESTEROL AND SUPERIOR TRIGLYCERIDE REDUCTION TO SIMVASTATIN 40MG IN HIGH-RISK PRIMARY HYPERCHOLESTEROLEMIA OR COMBINED DYSLIPIDEMIA

Neil Hounslow[1], D. Budinski[1], M. Eriksson[2]

[1]Medical Affairs, Kowa Research Europe Ltd, Wokingham, UK, [2]Karolinska University Hospital Huddinge, Stockholm, Sweden

This 2-phase study compared pitavastatin (PIT) and simvastatin (SIM) in patients with primary hypercholesterolemia or combined dyslipidemia and at least two CHD risk factors. Phase 1 followed a multicenter, double-blind, double-dummy, parallel-group design. Patients (n=355) were randomised to PIT 2mg titrated to 4mg at Week 4 or SIM 20mg titrated to 40mg at Week 4 taken once-daily for 12 weeks.

Reduction in LDL-C from baseline to Week 12 showed that PIT 4mg was non-inferior to SIM 40mg (44.0% and 43.8% respectively). PIT produced a greater reduction in TG (19.8% vs 14.8%; p=0.044) and increase in HDL-C (6.8% vs 4.5%; p=0.083) at Week 12 than SIM.

178 patients entered Phase 2, a 44-week double-blind, double-dummy extension. Patients assigned PIT 4mg continued with this dose. Patients assigned SIM 40mg continued or received SIM 80mg if they had not achieved target LDL-C by Week 8 of Phase 1.

LDL-C levels were 41.8% lower than the Phase 1 baseline with PIT and 41.4% lower with SIM 40/80. HDL-C progressively increased with PIT, but was more variable with SIM 40/80. Both groups showed >14% increases in HDL-C relative to Phase 1 baseline after 44 weeks. TG levels in both groups were approximately 12% lower than Phase 1 baseline after 44 weeks, but increased compared with the end of Phase 1.

The LDL-C lowering effect of PIT 4mg was non-inferior to SIM 40mg, whereas PIT 4mg produced a greater reduction in TG. Long-term treatment with PIT 4mg shows sustained efficacy and is well tolerated.

Memory Stick

TIME OF DAY DOSING DOES NOT INFLUENCE STEADY-STATE PHARMACOKINETICS OR LDL-C REDUCTION PRODUCED BY PITAVASTATIN

Roger E Morgan[1], Y. Inagaki[1], G.F. Arana[2], T. Hunt[3]

[1]Kowa Research Institute Inc, Morrisville, NC, [2]Kowa Pharmaceutical America Inc., Montgomery, AL, [3]PPD Development, Austin, TX, USA

Hepatic cholesterol synthesis is maximal when dietary intake is low. As a result, prescribing information for simvastatin, lovastatin and fluvastatin recommend evening dosing. This study aimed to determine the pharmacokinetic and pharmacodynamic effects of 14 days dosing of pitavastatin (PIT) 4 mg in the morning (AM) versus evening (PM). After a 28-day washout, patients crossed over to the alternative dosing time. Thirty-six healthy men or non-pregnant women, aged 18 to 45 years were assessed.

AM and PM dosing did not significantly influence steady-state AUC_{0-24} (198.71 and 222.46 ng.h/ml respectively), T_{max} (0.63 and 0.75 hours respectively) and C_{max} (82.31 and 84.37 ng/ml respectively). AM and PM dosing did not affect C_{max} (49.69 and 54.98 ng/ml respectively) or T_{max} (2.16 and 1.75 hours respectively) for pitavastatin lactone (PL), the inactive primary metabolite. However, mean AUC_{0-24} for PL increased slightly when PIT was administered in the PM compared to AM (468.80 versus 539.14 h.ng/mL respectively).

No significant differences between AM and PM dosing emerged for the effect on cholesterol. The reduction in LDL-C was 37.23% after AM dosing and 39.96% after PM dosing. The difference of least square means [DLSM] was 2.728, which is unlikely to be clinically significant. The reduction in total cholesterol was 28.77% and 31.27% respectively (DLSM 2.498).

In conclusion, the time of dosing did not affect pitavastatin's pharmacokinetics. No clinically significant differences were observed in pharmacodynamic parameters. These results support dosing of PIT at any time of day.

Memory Stick

EFFICACY OF SWITCHING FROM STATIN MONOTHERAPY TO EZETIMIBE/SIMVASTATIN 10/20 MG VERSUS ROSUVASTATIN 10MG IN HIGH-RISK PATIENTS ANALYZED BY BASELINE LDL-C

Philippe Brudi[1], M. Averna[2], M. Farnier[3], L. Missault[4], H. Vaverkova[5], L.M. Viigimaa[6], Q. Dong[7], A. Shah[7], W. Taggart[7], A. Johnson-Levonas[7]

[1]Merck Schering-Plough, Whitehouse Station, NJ, USA, [2]University of Palermo, Palermo, Italy, [3]Point Medical Rond Point de la Nation, Dijon, France, [4]St Jan Hospital, Brugge, Belgium, [5]Medical Faculty and University Hospital Olomouc, Olomouc, Czech Republic, [6]Tallinn University of Technology North-Estonia Regional Hospital, Tallinn, Estonia, [7]Merck Research Laboratories, Rahway, NJ, USA

This post-hoc analysis compared effects of switching from statins to ezetimibe/simvastatin 10/20 mg (EZE/SIMVA) vs rosuvastatin 10 mg (ROSUVA) in subgroups defined by LDL-C at baseline: Q1 (≤2.87 mmol/L) n=165, Q2 (>2.87 and ≤3.19 mmol/L) n=151, Q3 (>3.19 and ≤3.55 mmol/L) n=150 and Q4 (>3.55 mmol/L) n=152. High-risk patients with elevated LDL-C (≥2.59 and ≤4.92 mmol/L) despite statins entered an open-label period and received the same statin dose prior to randomization. Patients were randomized 1:1 to EZE/SIMVA 10/20 mg or ROSUVA 10 mg for 6 weeks. This analysis evaluated % change from baseline in lipids/lipoproteins in subgroups. Consistency of the treatment effect across subgroups was evaluated by testing for treatment×subgroup interaction without multiplicity adjustment. Treatment effects across the subgroups were generally consistent with the overall population (Table). EZE/SIMVA was more effective than ROSUVA at lowering LDL-C, TC, non-HDL-C and apo B in the overall population and subgroups. Irrespective of baseline LDL-C levels, switching from statin monotherapy to EZE/SIMVA 10/20 mg vs ROSUVA 10 mg provided superior reductions in LDL-C, TC, non-HDL-C and apo B.

Between-treatment differences (EZE/SIMVA-ROSUVA; 95% CI) in LS mean % change from baseline at study endpoint a

	Overall population	Q1	Q2	Q3	Q4	P-value for treatment x subgroup interaction
LDL-C (mmol/L)	-10.7 (-14.1, -7.3)*	-7.3 (-14.9, 0.3)	-8.1 (-15.8, -0.4	-8.8 (-15.0, -2.5)	-10.6 (-18.9, -2.3)	0.262
TC (mmol/L)	-7.2 (-9.6, -4.8)*	-5.2 (-10.3, 0.0)	-5.9 (-11.1, -0.7)	-6.8 (-11.5, -2.1)	-7.2 (-13.3, -1.2)	0.675
non-HDL-C (mmol/L)	-9.4 (-12.5, -6.3)*	-6.8 (-13.7, 0.1)	-7.6 (-14.9, -0.2)	-9.0 (-14.8, -3.3)	-9.3 (-17.3, -1.4)	0.693
apo B	-8.1 (-10.9, -5.3)*	-6.6 (-12.6, -0.6)	-5.9 (-12.0, 0.2)	-7.8 (-13.6, -1.9)	-4.9 (-12.5, 2.8)	0.873

a Last available post-baseline value collected during 6-wk active treatment period; *P<0.001 EZE/SIMVA vs ROSUVA

[Difference in LS mean % change from baseline]

Memory Stick

MORE UNIFORM CHOLESTEROLEMIC RESPONSE OF WOMEN VS MEN WHEN USING A DIVERSIFIED DRUG APPROACH TO HYPOLIPIDEMIC THERAPY

Giuliana Mombelli, N. Fabbri, S. Frasson, C. Baldo, V. De Vergori, M. Triolo, C.R. Sirtori

University Center for Dyslipidemias, Niguarda Hospital, Milano, Italy

Background and aim: The reduced cholesterolemic response in women, in particular in those at high cardiovascular risk (Mehner et al Eur J Clin Pharmacol 64, 815, 2008) has been noted in the accompanying report by our group, dealing with women and men treated only with statins.

Methods and results: In the present report we pooled data from patients in primary and secondary prevention (n=668), handled with a different drug approaches, based on the lipoprotein profile. Individuals with elevated triglyceridemia (> 200 mg/dl) were mainly prescribed either bezafibrate or fenofibrate, associated with satisfactory lipid outcomes in a previous study of ours (Mombelli et al, Int J Cardiol 133, 412, 2009); statins were mainly given in hypercholesterolemia. Statins were prescribed to 85.3 % of the series, vs 9.6 % for fibrates and lesser percentages for probucol, cholestyramine and ezetimibe. Differently from the case of statin only treated patients, this series showed less differences in response between sexes. Total cholesterol was reduced by 23.4±16.1% in males and 18.5±18.7% in females ($p< 0.001$) and the LDL-C reduction (-28.3±23.6% vs -18.5±52.0%) was again better in males ($p< 0.05$) but differences were less versus the statin only series. In addition, males had a modest mean triglyceride reduction, not significantly different from females (-10.2±57.5% and -0.3±92.1%); there were similar non significant rises of HDL-C.

Conclusion: An individualized drug approach to lipid lowering therapy in the two sexes, ie not only restricted to statins, provides an overall more uniform response versus statin only therapy, but clear differences remain.

Memory Stick

EFFECTS OF EZETIMIBE, INTIAL DOSES OF SATINS, AND ITS COMBINATION ON LIPIDS IN HIGH RISK PATIENTS WITH HYPERLIPIDEAMIA

Marina Zubareva, M. Tripoten', T. Rozhkova, E. Solovieva, T. Balakhonova, A. Rogoza, A. Susekov

FGU 'RKNPK Rosmedtekhnologii', Moscow, Russia

Aim: Aim of this study was to investigate the lipid effect of monotherapy of Ezetimibe (E), initial doses of simva -(S), atorva- (A), rosuvastatin (R) and its combinations in the treatment of high risk patients (pts) with primary hyperlipideamia (HL).

Methods: Sixty pts with LDL-C 3.0-4.9 mmol/l were randomized to take E 10 mg/day, S10, A10 or R 10 mg/day for the first 12 weeks. In 12 weeks time pts, who reached LDL-C< 2.5 mmol/l, continued assigned monotherapy, those pts with LDL > 2.5 were then taken combo therapy with E 10 mg/day for another 12 wks.

Results: Monotherapy with E10 resulted a -16% reduction in LDL-C, S10 -27%, A10 -34% and R10 -44% from baseline levels.

After 24 weeks of the treatment, in pts underwent therapy with combination E10 + S10 LDL-C reduction was -40%, in the group of E10+A10 -42%, E10+R10 -46%. Necessity in combo therapy (12 wks) was 53,3%. In monotherapy group S10, A10 and R10 (24 weeks) target LDLC- attainment was drop from 47% to 28%, less drops was seen in pts who have been taking A and R. Combination therapy E with S, A and R increase LDL-C attainment in average in 25%.

In conclusion, addition of E to inital doses of statins increase LDL-C goal attainment by 25%; pts taking synthetic statins both in mono and in the combined therapy kept LDL-C level control < 2.5 mmol/l. More than 50% of pts assigned to mono statin therapy would potentially need combination therapy.

Memory Stick

ARTERIAL FUNCTION AND STRUCTURE IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA. THE EFFECT OF ROSUVASTATIN

Constantina Masoura[1], C. Pitsavos[2], I. Skoumas[2], K. Aznaouridis[2], V. Metaxa[2], L. Papadimitriou[2], K. Psarros[2], C. Stefanadis[2]

[1]Lipid Clinic, [2]1st Department of Cardiology of Athens Madical School- Hippocration Hospital, Athens, Greece

Introduction: Familial combined hyperlipidemia (FCH) is related to increased cardiovascular risk. Arterial structure and function are predictors of outcomes.

Objectives: The effect of rosuvastatin on arterial function and subclinical atherosclerosis in FCH patients.

Methods: We studied 14 FCH (45 years), never treated, normotensives, non-diabetic, without cardiovascular disease. Arterial studies were performed before and 6 months after treatment with rosuvastatin 10 mg. Endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent nitrate-mediated dilatation (NMD) of the brachial artery, carotid-femoral pulse wave velocity (PWV, index of aortic stiffness), aortic augmentation index (AIx, marker of wave reflections), were measured non-invasively. Carotid atherosclerosis was evaluated by intima-media thickness (IMT) and plaques (IMT>1.5 mm) in right and left common carotid, bulb and internal carotid arteries by ultrasonography.

Results: Total cholesterol (300 vs.177 mg/dl), LDL-cholesterol (228 vs.108 mg/dl), triglycerides (249 vs.139 mg/dl) and apo-B (153vs.87 mg/dl) were reduced (P< 0,001, for all), after treatment. Rosuvastatin increased FMD (9.1 vs.3.5 %, P< 0.001) and FMD/NMD (0.63 vs.0.30, P< 0.001) and decreased PWV (7.9 vs.8.5 m/sec, P< 0.001). We observed no change in AIx (17.3 vs.16.9%, P=NS). Rosuvastatin significantly reduced the mean number of carotid plaques (1.4 vs.2.0, P=0.005) and IMT of common carotid (0.77 vs.0.86 mm, P=0.044), bulb (1.17 vs.1.28mm, P=0.005) and internal carotid arteries (0.94 vs.1.07 mm, P=0.003). Multivariable analysis showed that the above arterial changes were not fully accounted for by the decrease in blood lipids.

Conclusions: In FCH patients, rosuvastatin improves endothelial function and aortic stiffness and results in regression of subclinical carotid atherosclerosis, independently of lipid reduction.

Memory Stick

EVALUATION OF EFFECTIVENESS OF THE COMBINED TREATMENT WITH ATORVASTATIN AND EZETIMIBE IN PATIENTS WITH CHD

Ketevan Kapanadze, N. Kipshidze

Acad.N.Kipshidze National Center of Therapy, Tbilisi, Georgia

Background: The risk of progression of the atherosclerotic process to coronary heart disease increases progressively with the increasing levels of total cholesterol (T-C) and especially with low density lipoprotein cholesterol (LDL-C). Data of clinical trials indicate that decrease in LDL-C levels significantly reduces the risk of CHD development.

The aim of the study was to study the efficacy of the combined treatment with Atorvastatin and Ezetimibe in patients with CHD.

Materials and methods: We have studied efficacy of Atorvastatin treatment only and also in combination with Ezetimibe. It was observed, that combined therapy is four times more efficient and gives us opportunity to prevent the side effects of statins and strengthen their effectiveness. We studied 62 patients (41 males/21 females, age range from 42 to 79 years). Mean indices of T-C, HDL-C, LDL-C and TG were: 287.0mg/dl, 32.6mg/dl, 193.9mg/dl and 192.0 mg/dl, correspondingly. Patients were treated with 10 mg Ezetimibe and 20 mg Atorvastatin, during 8 weeks.

Results: Analyses of the data obtained revealed statistically evident difference between the results obtained pre-and post-treatment initiation and target levels of the lipid profile were reached in 78% of patients. Clinical improvement of patients' condition was evaluated via the decrease of angina pectoris attacks and nitro-glycerine consumption.

Conclusion: Hence, in order to avoid the side effects of statins (caused with high doses) and at the same time reach the target levels of cholesterol, which predicts the discontinuation of atherosclerotic process, combined therapy and dual inhibition with Ezetimibe+Statins gives chance atherosclerosis progression to be stopped.

Memory Stick

DETERMINING THE OXIDATIVE BURDEN IN PATIENTS WITH CORONARY ARTERY DISEASE USING A NOVEL DIRECT ACTIVATOR OF HEME-FREE/OXIDIZED SGC

Ingo Ahrens[1], J. Habersberger[2], H. Qian[2], J.-P. Stasch[3], C. Bode[1], H. Schmidt[4], K. Peter[2]

[1]Cardiology and Angiology, University Hospital Freiburg, Freiburg im Breisgau, Germany, [2]Atherothrombosis & Vascular Biology, Baker IDI Heart & Diabetes Institute, Melbourne, VIC, Australia, [3]Cardiology Research, Pharma Research Centre, Bayer Schering Pharma AG, Wuppertal, Germany, [4]Department of Pharmacology, Monash University, Melboure, VIC, Australia

Objective: This study assessed oxidative burden in patients with coronary artery disease (CAD) using the oxidation state of platelet soluble guanylate cyclase (sGC) as a proxy for endogenous oxidative processes.The production of reactive oxygen species (ROS) is thought to be a key initial event in atherosclerosis. However, direct assessment of ROS is difficult as sample processing may dramatically alter results.

Methods: Platelets were obtained from patients with and without CAD confirmed by angiography. Platelets were treated with a sGC oxidizing agent (ODQ) or vehicle and incubated with the sGC activator Cinaciguat (BAY 58-2667). Platelet activation was assessed by flow cytometry and phosphorylation of platelet VASP at Ser^{239} was determined to assess sGC activity.

Results: ADP-induced platelet activation was inhibited in the presence of Cinaciguat and was further impaired with the addition of ODQ. Calculating the difference (Cinaciguat) - (ODQ+Cinaciguat) demonstrated lower values in patients with CAD (17.19 patients vs 33.99 controls, p=0.016) suggesting a greater amount of oxidised sGC *in vivo*. In parallel VASP phosphorylation was assessed and the ratio of *ex vivo* oxidised sGC/native sGC determined. This was lower in patients with CAD (1.44 patients vs 2.93 controls, p=0.041) showing that patients with CAD respond more selectively to the haem-independent sGC activator Cinaciguat.

Conclusion: We present a novel flow cytometry assay that reveals a higher oxidative burden in patients with CAD compared to patients without CAD. This assay may allow assessing the patient's response to direct sGC activators, enabling individualized pharmacotherapy with this new class of drugs.

Memory Stick

A NEW CONTOUR ANALYSIS OF PHOTOPLETHYSMOGRAPHIC PULSE MEASURED AT THE FINGER

Hsien-Tsai Wu[1], B.-S. Lin[1], C.-C. Liu[1], A.-B. Liu[2]

[1]Electrical Engineering, National Dong Hwa University, Haulien, [2]Department of Neurology, Tzu-Chi General Hospital, Hualien, Taiwan R.O.C.

Cardiovascular disease (CVD) is currently the biggest single cause of mortality in the developed world, hence, the early detection of its onset is vital for effective prevention therapies. A volume pulse is utilized for assessing arterial properties because of its simplicity and ease of use. As with the pressure pulse, the contour of the digital volume pulse is sensitive to changes in arterial tone induced by vasoactive drugs and is influenced by ageing and large artery stiffness. Measurements taken directly from the digital volume pulse or from its crest time, i.e., the time from the foot of the waveform to its peak. Furthermore, we also found that the interval from footpoint to the second peak of the pulse tends to fix and crest time is increasing with age.

In this study, we followed the methods of an index of large artery stiffness (SI), to formulate some new indices to assess the stiffness of artery. Thus new contour analysis of the digital volume pulse provides a simple, reproducible, non-invasive measure of large artery stiffness.

Memory Stick

THE INFLUENCE OF RAMIPRIL (AMPRILAN) ON THE INFLAMMATION MARKERS AND THE IMMEDIATE PROGNOSIS OF MYOCARDIAL INFARCTION

Arcady Vertkin, A. Skotnikov

Moscow State Medical Stomatological University, Moscow, Russia

Aim: The aim of the research was to find out the advantages of ramipril (amprilan) in the influence on the inflammation markers and the terminal point (death, recurrent myocardial infarction, stroke) within 1 and 3 months after the myocardial infarction.

Material and methods: The concentration of C-reactive protein, total cholesterol, triglycerides, cholesterol of high-density lipoproteins and cholesterol of low-density lipoproteins was determined in all the patients (n=60).

Results: The amprilan therapy reduced the frequency of angina pectoris emergence in the post infarction period (65% against 36% in the control group), brought about the deceleration of the hypertrophy intensity growth as well as the mass of the myocardial left ventricle, and reduction of C-reactive protein concentration in the blood (by 87,6% against 36,4%). In the amprilan group the level of total cholesterol was reduced by 8,1% against 4,9%, of triglycerides by 10,4% against 7,3% and cholesterol of low-density lipoproteins by 7,8% against 9,8%, while the cholesterol of high-density lipoproteins content raised by 8,4% against 2,9% in the control group accordingly. No recurrent cases of myocardial infarction and, stroke were registered in the amprilan group.

Conclusion: Amprilan, administered within the first 24 hours of the acute coronary syndrome development, reduces the frequency of angina pectoris emergence in the post infarction period, lowers the C-reactive protein, depresses the inflammation processes in myocardium and reduces the level of its remodeling and also lowers the level of total cholesterol, improving the patients prognosis and minimizing the risk of fatal cardial and cerebral-vascular events development.

Memory Stick

PREVALENCE OF SIGNIFICANT CAROTID STENOSIS IN IRANIAN PATIENTS WITH PERIPHERAL VASCULAR DISEASE

Abolhassan Shakeri Bavil, M. Nemati, S. Daneshmand

Tabriz University of Medical Sciences, Tabriz, Iran

Background and aims: The aim of this study was to determine the prevalence of severe carotid artery stenosis in patients with peripheral vascular disease (PVD).

Methods: 120 Consecutive patients with angiographically proven PVD undervent prospective screening for the presence of carotid stenosis with color Doppler ultrasonography. This study was done in Imam Khomeini Hospital of Tabriz, Iran.

Results: The mean age of the patients was 58.52±11.04 years, and there was 81 (85.3%) men and 14 (14.7%) women. 26 percent of the patients had a history of smoking, 6% had a history of coronary artery disease, 6% had hypertension, 10% had diabetes. The prevalence of risk factors were similar in patients and control group.Carotid artery stenosis was present in 4.2% of the patients and 1% in control group. The prevalence in patients was twice than in control group but it was not statistically significant ($p>0.05$).

Conclusion: Iranian patients with PVD show low prevalence of severe carotid artery stenosis. Age is an important determinant of carotid disease, the mean age of our patients was found to be lower than many other studies. Iranian patients seem to involve PVD in younger age probably as a result of absence of regular exercise in their daily program.

Memory Stick

KEY LABORATORY DIAGNOSTIC BIOMARKERS OF CORONARY ATHEROSCLEROSIS IN SIBERIA

Yulia Ragino[1], A. Chernjavski[2], N. Eremenko[3], E. Schahtshneider[3], Y. Polonskaja[3], S. Tcimbal[2], M. Ivanova[3], M. Voevoda[3]

[1]Laboratory of Biochemistry, Institute of Internal Medicine SB RAMS, [2]Research State of Circulation Pathology, Federal Agency of Public Health and Social Development, [3]Institute of Internal Medicine SB RAMS, Novosibirsk, Russia

Laboratory lipid and lipoprotein biomarkers (cholesterol, CH, triglycerides, TG, low-density and high-density cholesterol, LDL-CH, HDL-CH, apolipoproteins B and A1, apoB, apoA1), carbohydrate biomarkers (plasma glucose, basal insulin), high sensitive C-reactive protein (hsCRP) and oxidative biomarkers (basal level of lipid peroxidation (LPO) products in LDL, LDL resistance to oxidation *in vitro*, oxidative modification of apoLDL and level of alpha-tocopherol and retinol in LDL) were studied in 388 men 42-70 years old, including 96 citizens of Western Siberia with coronaroangiographia documented coronary atherosclerosis and coronary heart disease (CHD) and 292 men of population simple of citizens of Novosibirsk. In 44 men of population group the CHD was determined by standardized epidemiological criterions and methods, including electrocardiographic Minnesota code. Finally, the main group of study was formed from 140 men with coronary atherosclerosis and CHD and the control group - from 248 men without CHD. Increased levels of blood TG, apoB, basal insulin, hsCRP and basal level of LPO products in LDL and decreased levels of HDL-CH, apoA1 and LDL resistance to oxidation were determined in the main group compared to control group. Significant associations and correlations of coronary atherosclerosis and CHD with laboratory diagnostic biomarkers like the blood levels of HDL-CH, TG, apoB, apoA1, basal insulin, hsCRP and basal level of LPO products in LDL and LDL resistance to oxidation were also revealed. In conclusion, this biochemical panel, including indicated biomarkers, may be important for laboratory diagnostics of coronary atherosclerosis in Siberia. The study was supported by Grant of RFBI 09-04-00374.

Memory Stick

RANDOMIZED TRIAL OF LIPID APHERESIS IN PATIENTS WITH ELEVATED ISOLATED LIPOPROTEIN(A) CONCENTRATIONS: STUDY DESIGN OF THE ELAILA TRIAL

Heiner Berthold[1], A. Rosada[1], C. Erley[2], I. Gouni-Berthold[3], M. Leitzmann[4], K. Parhofer[5], G. Schmitz[6], J. Thiery[7], A. Vogt[8], E. Steinhagen-Thiessen[1], The ELAILa Study Group

[1]Lipid Clinic at the Interdisciplinary Metabolism Center, Charité University Medicine Berlin, [2]Dept. of Internal Medicine II (Nephrology), St. Joseph Hospital Berlin-Tempelhof, Berlin, [3]Dept. of Internal Medicine II, University of Cologne, Cologne, [4]Dept. of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, [5]Dept. of Internal Medicine II, LM University of Munich, Munich-Grosshadern, [6]Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Regensburg, [7]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Leipzig, [8]Medical Policlinic, LM University of Munich, Munich-Innenstadt, Germany

Introduction: Lipid apheresis is the only effective measure to reduce markedly elevated concentrations of lipoprotein(a). However, there is no high-level evidence that Lp(a) apheresis decreases cardiovascular events. The German Federal Joint Committee (G-BA) has made Lp(a) apheresis a reimbursable treatment option within the compulsory health insurance system in patients with isolated Lp(a) elevation >60 mg/dl, normal LDL-cholesterol concentrations and progressive cardiovascular disease. At the same time, G-BA mandated a controlled prospective trial to investigate if the procedure is able to decrease cardiovascular events.

Design: The ELAILa study group devised an investigator-initiated trial as a hybrid 2-branch/2-group study. Primarily the study will investigate eligible patients randomized to either apheresis treatment or standard care (RCT). If patients decline being randomized they will be asked to participate in an observational trial (OT). Within the OT they may be treated with apheresis or standard care. The primary outcome is a composite endpoint consisting of non-fatal myocardial infarction, interventional therapeutic procedure (PTCA), coronary bypass surgery, non-fatal cerebrovascular accident, hospitalization due to acute coronary syndrome, or death from cardiovascular causes.

Objective: The current paper presents the study design and further details of this trial. The objective is to investigate whether lipoprotein(a) apheresis prevents cardiovascular events. Furthermore, safety, tolerability and quality of life will be investigated. The trial will be powered to detect a relative risk reduction in the primary endpoint of 50 percent at $P< 0.05$ with a power of 80 percent. Approx. 135 patients will be randomized. Recruitment and follow-up will be approx. five years.

Memory Stick

MORUS ALBA EXTRACT AND CURCUMIN REDUCE RESISTIN INDUCED-HUMAN ENDOTHELIAL CELLS ACTIVATION BY A MECHANISM INVOLVING P38 MAPK

Monica Madalina Pirvulescu[1], D. Stan[1], A.-M. Gan[1], V. Simion[1], C. Ionescu-Targoviste[2], I. Manduteanu[1]

[1]Institute of Cellular Biology and Pathology 'Nicolae Simionescu', [2]The National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

The extract from Morus Alba (MA) leaves is used in the Chinese traditional medicine as a hypoglycemic agent. Recent data indicate that MA has also antioxidant and anti-inflammatory effects but the mechanisms involved are not well known. Curcumin has been used in a variety of diseases in traditional medicine and has anti-inflammatory, antioxidant and cardiovascular protective effects. Resistin is a newly discovered cytokine recognized to be a significant local and systemic modulator of inflammation. In the present study we evaluated the anti-inflammatory potential of the MA extract and curcumin on endothelial cells (EC) stimulated by resistin. To this purpose, human EC in culture (EAhy926 cell line) were exposed to 100 ng/ml resistin in the absence or presence of therapeutic doses of MA (400µg/ml) and curcumin (20 µM) and the adhesion of monocytes, the expression of cell adhesion molecules P-selectin and fractalkine and the activation of p38MAPK were tested. Adhesion assays, RT-PCR and western blot analysis revealed that MA extract and curcumin reduced monocyte adhesion , P-selectin and fractalkine expression and p38MAPK activation induced by resistin in EC. Together these data indicate a benefic anti-inflammatory effect of MA and curcumin on human endothelial cells and that p38 MAPK pathway is involved in the mechanism of action of these drugs.

Funding: This work was supported by Romanian Academy and the Ministry of Education and Research-CNMP Grant No.41037.

Memory Stick

MULTIPLE CORONARY ARTERY ANEURYSMS IN SUBJECT CARRIER ASP9/ASN LIPOPROTEIN LIPASE MUTATION

M. Puntoni[1], **Francesco Sbrana**[2], F. Bigazzi[2], B. Pennato[3], B. dal Pino[1], D. Iori[2], S. Bertolini[4], P. Marraccini[2], D. Rovai[2], T. Sampietro[2]

[1]CNR Institute of Clinical Physiology, [2]Gabriele Monasterio CNR Regione Toscana Foundation of Pisa, [3]Scuola Superiore Sant'Anna, Pisa, [4]Departement of Medicine, University of Genova, Genoa, Italy

His lipid and apolipoprotein profile was characterized mainly by high triglycerides (TG) (482 mg/dl) and low HDL-cholesterol (HDL) (32 mg/dl). Apolipoprotein (Apo) plasma concentrations were: 6.61 mg/dl for ApoE, 99.1 mg/dl for Apo A1, 112.4 mg/dl for Apo B; total cholesterol and Lipoprotein(a) values were respectively 205 mg/dl and 9.6 mg/dl.

The patient was free of other diseases or risk factor as arterial hypertension or diabetes.

Because of exertional chest pain and EKG stress test positive for myocardial ischemia, a coronary angiogram was performed.

The test revealed diffuse dilatation of the circumflex artery, multiple aneurysmatic dilatations at the proximal and mid portion of the left anterior descending artery and at the proximal portion of right coronary while stenotic lesions were completely absent.

Lipid-lipoprotein patient profile was suggestive of Lipoprotein Lipase (LPL) mutation, which was indeed identified to be a Asp9/Asn mutation.

Heterozygote carriers of Asp9/Asn LPL mutation are known to be prone to premature atherosclerotic coronary disease. In the case here reported atherosclerotic process determined an aneurysmatic pattern rather than the more common, and expected on the basis of clinical presentation, stenotic one.

Memory Stick

INFLUENCE OF SMOKING AND DIABETES MELLITUS TYPE 2 TO THE DEVELOPMENT OF CORONARY DISEASE

Branko Sreckovic[1], V. Dimitrijevic-Sreckovic[2], J. Perunicic[3], P. Djordjevic[2], E. Colak[4], I. Soldatovic[5], M. Gajic[5]

[1]Internal Medicine, Clinical Center Bezanijska Kosa, [2]Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [3]Institute of Cardiovascular Diseases, [4]Institute of Medical Biochemistry, [5]Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia

Aims: To examine the influence of smoking and diabetes mellitus type 2 (DM2) as coronary disease (CD) essential risk factors, as well as other risk factors, on the occurrence of CD.

Material and methods: The study included 141 patients, aged 40 to 70: Group I (no DM2, no CD) - control group, Group II (no DM2, miocardial infarction), Group III (DM2, no CD) and Group IV (DM2, CD). The following parameters were measured: waist circumference (WC), body mass index (BMI), blood pressure, lipid status and glycoregulation (determined through HbA1C by spectrophotometry). Insulin sensitivity was determined by HOMA IR.

Results: Obesity: by BMI (I-22.8±3.2, II-27.8±3.8, III-29.4±6.0, IV-30.6±4.4 kg/m2); by WC (I-76.7±8.3, II-98.2±12.3, III-95.9±15.2, IV-100.4±12.4 cm). Hypertension: I-21.4%, II-66.7%, III- 72%, IV-82%. Hyperlipoproteinemia (HLP): I-5%, II-75%, III-52%, IV-75%. Values of HbA1C: I-5,2±0.2, II-5.1± 0.5, III-7.5±1.5, IV-7.9±1.6%. Value of HOMA IR was highest in Group IV (diabetics with CD). Kruskal-Wallis test indicated a statistically highly important difference $p< 0.01$ between groups (I-2.7±1.1, II-5.1±3.8, III-8,1-4.6, I IV-13.0±6.6 mmol/µU/ml). Influence of smoking on the occurrence of CD, expressed by packs number per annum, was 40.5±24.6 and 24.5±29.0 in CD and non-CD patients, respectively. Mann-Whitney test found a statistically important difference between groups regarding packs number per annum ($p< 0,05$).

Conclusion: Individuals with DM have 2.56 times higher chances to become CD patients compared to non-diabetics. Numbers of cigarettes smoked and smoking years, expressed in packs number per annum, showed an increase of CD directly related to packs number p.a. and not related to patients age.

Memory Stick

THE INFLUENCE OF ONE-YEAR TRAETMENT WITH ATORVASTATIN, ROSUVASTATIN AND SIMVASTATIN ON INTIMA MEDIA THICKNESS IN PATIENTS WITH HEART ISCHEMIC DISEAS

Manana Akhvlediani[1], E. Vorobiova[1], T. Vakhtangadze[2], M. Emukhvari[1], M. Balavadze[2], T. Kvantaliani[3]

[1]Laboratory of Clinical Biochemistry, [2]Department of Functional Diagnostics, Institute of Clinical Medicine, [3]Department of Functional Diagnostics, Institute of Cardiology, Tbilisi, Georgia

Objective: To study the effect of one-year treatment of Atorvastatin, Rosuvastatin and Simvastatin on Intima media Thickness (IMT), interleukins,Hs- CRP, Protein C, D dimmers and SFMC in patients with ischemic heart desease.

Methods: 146 patients with ischemic heart desease (Tch>6.2 mmol/l) were investigated. The mean age of the patients varied within 55.4±10.7. They were divided into 3 groups. Group I (N=84) was treated with 20 mg Atorvastatin(Liprimar); Group II (N=10) with 10 mg Rosuvastatin (Krestor); Group III (N=52) with 40 mg Simvastatin (Zokor).Uultrasound scanning of carotid arteries was perfomed for each subject. The interleukins(IL-1, IL-6,) lipids, Hs- CRP, ProteinC and D dimers were defined as well.

Results: Under the treatment perfomed, the Tch($P< 0.01$) and LDL($P< 0.05$) were noted to decrease in the three groups, while HDL increased ($P< 0.05$). The same tendency to decrease the mean values of IL-1,IL-6 Hs- CR, SFMC and D dimers is observed. IMT decreased by 24.2%, 26.1% and 23.4% relatively. One should mention that despite a well-defined positive dynamics in the above three groups, it was more expressed in group III.

Conclusion: One-year treatment with Atorvastatin, Rosuvastatin and Simvastatin resulted in the normalization of lipids, as well as in that of interleukins, Hs-CRP, Ddimers, SFMC and Protein C. But IMT decreased by 24-26%. Due to high hypolipidemic, untiinfammatory and antithrombotic effects of the therapy, it is necessary to use them for a long time in the primary and secondary prophilaxis of the heart ischemic diseases.

Memory Stick

PROTEOMIC ANALYSIS FOR BIOMARKER OF VULNERABLE PLAQUE IN PATIENTS WITH UNSTABLE ANGINA

Hyuck Moon Kwon[1], K.H. Jung[2], J.Y. Kim[1], B.K. Lee[1], P.K. Min[1], H.S. Kim[3]

[1]Internal Medicine, Cardiology, Gangnam Severance Hospital, Yonsei University, Seoul, [2]Biochemistry, CHA Medical University, Sungnam, [3]Internal Medicine, Kyungkido Paju Medical Center, Paju, Republic of Korea

Coronary artery disease (CAD) is a major cause of mortality and morbidity. Acute coronary syndromes (ACS), such as unstable angina (UA), acute myocardial infarction, and sudden cardiac death, are commonly associated with the presence of vulnerable plaques in coronary arteries. The discovery of reliable biomarker for early detection of UA could guide better clinical evaluation and preventive treatment. To identify the differentially expressed proteins in plasma of UA patients, a total of 15 patients plasma and 15 control plasma were comparatively analyzed by proteomic analysis using 2-DE and consecutive MALDI-TOF-MS. In total, 32 spots of 2-DE showed significantly difference in protein expression between unstable angina and normal control. Among them, 19 proteins were highly expressed in UA. After validation with ELISA, beta 2- glycoprotein 1 (b2GP1) or apolipoprotein H was found to be the most obviously overexpressed protein in UA among the selected proteins. Our results suggest that b2GP1 may be a useful diagnostic and prognostic biomarker in UA.

Memory Stick

REDUCED MYOCARDIAL GLUCOSE UTILIZATION AFTER FASTING AND HEPARIN INJECTION IN RATS

Ralf Bergmann, J. Pietzsch

Institut für Radiopharmazie, Forschungszentrum Dresden-Rossendorf, Dresden, Germany

Quantitative assessment of inflammatory processes in the vasculature using [^{18}F]fluorodeoxyglucose (FDG) and small animal positron-emission-tomography (PET) requires low glucose uptake in surrounding tissues to obtain low background in PET images. We investigated the effect of food supply and heparin on FDG uptake in both the myocardium and, as reference, the brain by biodistribution (tissue specific glucose consumption) and dynamic PET (K_m of the metabolic rate of glucose) experiments in male Wistar rats.

Myocardial and brain glucose consumption were measured at 5 and 60 min after single intravenous (iv) FDG bolus in four groups: fed (control; free access to water and standard food), fasting (24 hours), fed+heparin (iv, 10 min before FDG) and fasting+heparin.

Fasting resulted in decreased glucose myocardial uptake (standard uptake value (SUV), mean±SD; 4.5±0.7 vs 1.9±1.1, $P < 0.0001$). Additional application of heparin further decreased glucose uptake to 0.8±0.3 ($P < 0.05$). Myocardial K_m was compared between fed and fasting group. It was decreased from 0.038±0.016 (fed) to 0.005±0.002 (fasting) mL/g/min $P < 0.0001$. Brain uptake was increased both in fasting and fasting+heparin group.

Fasting, resulting in lower blood glucose levels, in rats acutely reduces myocardial glucose uptake. Addition of heparin enhances this effect, probably, via activation of lipoprotein lipase and subsequent increment in myocardial free fatty acid supply.

In conclusion, fasting with or without heparin supposed to be a suitable prerequisite for low background imaging, e.g., inflammatory processes in thoracic region.

Memory Stick

IMPACT OF PLAQUE HAEMORRHAGE ON PLAQUE STRESS IN PATIENTS WITH CAROTID ARTERY DISEASE- A PATIENT-SPECIFIC MAGNETIC RESONANCE IMAGING STUDY

Umar Sadat, Z.Z. Teng, M.J. Graves, J.H. Gillard

University of Cambridge, Cambridge, UK

Introduction: High resolution magnetic resonance (MR) imaging can be used to assess the atherosclerotic plaque component-dependent biomechanical stresses.

Aims: To assess the effect of plaque haemorrhage (PH) and age of PH on the plaque stresses by performing patient-specific MR-based computational simulations.

Methods: 27 symptomatic patients and 23 asymptomatic patients underwent high resolution MR imaging of their atherosclerotic carotid arteries. Axial T_1, T_2-weighted, STIR and proton density-weighted images covering the entire carotid plaque were acquired. Manual segmentation of plaque components was done. Patients with MR evidence of PH were only included for this study. Critical stress was generated using finite element method and solved in ADINA 8.5 (ADINA, Inc.).

Results: The median critical stress of symptomatic patients with fresh PH was 159kPa (IQR: 114-253). When the simulation was repeated with fresh PH replaced with lipid pool, the stress was 118kPa (IQR: 79 to 189) (p=0.001), showing that fresh PH caused a 26% (IQR: 13-50) increase in the critical stress from baseline. The simulation performed with chronic haemorrhage replacing fresh PH gave a stress of 118kP (IQR: 79-189), (p=0.001). This indicated a 30% (IQR: 19-44) reduction in the critical stress. The critical stress of asymptomatic patients with chronic PH was 145kPa (IQR: 104-188). The simulation performed with chronic PH replaced with lipid pool produced a critical stress of 152kPa (IQR: 109-204). There was no significant difference between these two states (p=0.65).

Conclusions: Age of the plaque haemorrhage affects biomechanical stresses within atherosclerotic plaques,with fresh PH significantly increasing the plaque stresses .

Memory Stick

AN IMPROVEMENT OF ENDOTHELIAL DYSFUNCTION MARKERS IN CHRONIC KIDNEY DISEASE SIMVASTATIN/EZETIMIBE COMBINED THERAPY

Ciriaco Carru[1], G. Loriga[2], G. Farre[2], A. Zinellu[1], S. Sotgia[1], B. Scanu[1], E. Pisanu[1], M. Sanna[1], L. Deiana[1], A. Satta[2]

[1]Biomedical Sciences, [2]Department of Internal Medicine, University of Sassari, Sassari, Italy

Although previous studies suggest that treatment of dyslipidemia reduces mortality and morbidity that are associated with cardiovascular disease, the role of lipid lowering treatment in vascular protection is not still understood in patients with chronic kidney disease (CKD). In large randomized clinical trials, co-administration of ezetimibe with statin proved to be more effective in lowering cholesterol levels than treatment with statin alone. In a 12-month observational period, we assessed the effects of simvastatin/ezetimibe combined therapy (20/10 mg/day or 40/10 mg/day) in lipid parameters (total cholesterol; low-density lipoprotein cholesterol, LDL-C; high-density lipoprotein cholesterol, HDL-C; triglycerides), markers of endothelial dysfunction (asymmetrical dimethylarginine, ADMA) and renal outcomes in ten patients with CKD.

We observed a decrease of 38.7% in total cholesterol, 56% in LDL-C, 17.4% in triglycerides, and an important reduction in LDL/HDL ratio (3.45 1.6 at baseline vs 1.41 0.5 after 12 months, $p = 0.001$), with a 5% increase in HDL-C. Concomitantly, ADMA plasma levels significantly decreased (0.71 0.12 at baseline vs 0.63 0.08 micromol/L after 12 months, $p = 0.015$), and there was a strong direct correlation with total cholesterol ($r = 0.67$, $p = 0.033$), LDL-C ($r = 0.75$, $p = 0.013$) and LDL/HDL ratio ($r = 0.73$, $p = 0.016$). Renal parameters did not changed from baseline in a significant extent. Our data suggest that Simvastatin/ezetimibe combined therapy is a valid and safe therapy by an improvement in lipid profile, could translate in the correction of endothelial dysfunction, potentially cardioprotective in the long-term.

Memory Stick

SHORT TERM (3MONTHS)EVALUATION OF RENAL ARTERY STENTIN AND ANGIOPELASTY IN PATIENTS WITH HYPERTENSION AND RENAL ARTERY STENOSIS

M. Shabestari[1], F. Sharifipour[2], M. Dadollahi[2], **Meisam Haghmoradi**[2]

[1]Cardiology Department of Imam Reza Hospital, [2]Mashhad University of Medical Sciences, Mashhad, Iran

Background: Renal artery stenosis comprise both atherosclerotic renovascular disease and fibromuscular dysplasia which is more common in female and may be associated with refractory hypertension, acute 'flash' pulmonary edema and renal failure. To assess the procedural and short-term safety and efficacy of renal artery stenting and its effect on blood pressure, antihypertensive medication usage and serum creatinine.

Methods: All patients referred for renal artery stenting were entered into a prospectively collected database. Systolic and diastolic blood pressure, number of antihypertensive medications, and serum creatinine were recorded. Patients were followed-up at least three months post-procedure.

Results: fifty-two patients underwent renal arteriography. The procedural success rate was 100% with no procedural mortality. There were no complications.

Follow-up was 3 months. Any patients were not lost to follow-up. There were no deaths. Significant fall in systolic blood pressure (BP) from 179mmHg pre-procedure to 145mmHg at short-term post-procedure Follow-up ($p < 0.0001$). The clinical restenosis rate was 3%. Renal function remained stable in 65% and decrease of Cr was seen in 15%. There was a significant decrease in the number of antihypertensive medications from 2.9 to 1.6.

Conclusion: Renal artery stenting is safe and appears effective for the treatment of clinically significant renal artery stenosis.

Memory Stick

REVASCULARIZATION STRATEGIES AFTER THE SYNTAX TRIAL: THE GLOBAL PICTURE AND A SINGLE CENTER EXPERIENCE

Lenard Conradi[1], H. Treede[1], O. Deutsch[1], P. Marcsek[1], M. Ismail[1], T. Meinertz[2], H. Reichenspurner[1]

[1]Department of Cardiovascular Surgery, [2]Department of Cardiology, University Heart Center Hamburg, Hamburg, Germany

Objectives: Despite established guidelines for treatment of coronary artery disease (CAD) by coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI), clinical practice differs substantially with the most complex coronary lesions targeted by PCI today. From the SYNTAX trial, data of state-of-the-art percutaneous and surgical revascularization strategies in three-vessel and/or left main CAD are now available to guide clinical decision-making.

Methods: We analyzed 2-year data from the multi-center SYNTAX trial and compared results with our experience as participating center.

Results: The primary endpoint of non-inferiority for PCI regarding major adverse cardiac and cerebrovascular events was not met. At two years, CABG was significantly superior in terms of repeat revascularization (8.6vs17.4%,p< 0.001), cardiac death (2.7vs.4.5%,p=0.04) or myocardial infarction (3.3vs.5.9%,p=0.01). At our center, 30 patients were randomized to undergo CABG or PCI (n=15 each). As a major difference to the general study, where PCI was superior to CABG regarding stroke rate (1.4vs2.8%,p=0.03), no patient in the CABG cohort suffered a stroke until two years of follow-up.

Conclusions: Because of significant advantages regarding repeat revascularization, myocardial infarction and cardiac death, CABG should remain the primary treatment modality in three-vessel and/or left main CAD. In our experience, CABG is as safe as PCI regarding stroke rate, possibly due to use of off-pump coronary bypass grafting and avoidance of aortic cross-clamping. However, there is a role for PCI in patients with low SYNTAX scores, severe comorbidities or in ongoing myocardial injury. Interdisciplinary evaluation of patients is paramount for successful treatment of complex CAD.

Memory Stick

DEVELOPMENT AND EVALUATION OF A NOVEL POLY-L-LACTIC ACID / CAPROLACTONE COPOLYMER CORONARY STENT MANUFACTURED BY SELECTIVE LASER MELTING

Felix Vogt[1], C. Flege[1], S. Hoeges[2], M. Borinski[1], V.A. Schulte[3], R. Hoffmann[1], N. Marx[1], R. Poprawe[2], K. Wissenbach[4], R. Blindt[1]

[1]Cardiology, Pneumology and Angiology, University Hospital, RWTH Aachen, [2]Lehrstuhl für Lasertechnik, [3]DWI an der RWTH Aachen e.V., RWTH Aachen University, [4]Fraunhofer-Institut für Lasertechnik, Fraunhofer-Gesellschaft, Aachen, Germany

Objective: Stent-based therapies in coronary intervention are associated with substantial complication rates. Biodegradable stents might reduce the foreign-body reaction. Here, feasibility of processing biodegradable polymer powders to stent structures by the novel manufacturing technology selective laser melting (SLM) and biocompatibility of SLM-manufactured polymer samples were assessed.

Methods: Cell culture samples for in vitro tests were manufactured by SLM from poly-L-lactic-acid (PLLA) and PLLA copolymer (PLLA/co-poly-ε-Caprolactone [70/30]). Biocompatibility was evaluated for human artery smooth muscle cells (SMC), human umbilical vein endothelial cells (HUVEC), and endothelial progenitor cells (EPC). Scanning electron microscopy (SEM) to determine cellular morphology, fluorescence microscopy to assess number of adhering cells, and vitality tests were performed. Furthermore, tailored manufacturing of 3-dimensional structures was evaluated by modification of SLM processing.

Results: By the SLM method, near complete polymer density was gained. Biocompatibility evaluation of SLM-processed PLLA and PLLA copolymer evidenced pronounced adherence of HUVEC and EPC. SLM-PLLA and PLLA copolymer did not impair SMC, EPC, and HUVEC vitality. As evidenced by SEM and fluorescence microscopy, adhesive capacity of SMC, EPC, and HUVEC on SLM-PLLA and PLLA copolymer samples was comparable to vicryl. By computer aided design, initial SLM-manufactured PLLA copolymer tubular structures were adapted to generate prototypic coronary stents with reliable balloon-expandability by standard interventional equipment.

Conclusion: PLLA polymers displayed pertinent biocompatibility and adhesive properties. The SLM manufacturing technique enabled processing of PLLA copolymer powder to functional 3-dimensional stent geometries. Combined with incorporation of drugs, SLM-processing of PLLA copolymer is a promising strategy for biodegradable drug eluting coronary stents.

Memory Stick

ANTIATHEROSCLEROTIC EFFECT OF ACACIA SENEGAL SEED EXTRACT IN CHOLESTEROL-FED RABBITS. ASHOK PUROHIT AND HEERA RAM* DEPARMENT OF ZOOLOGY, JNV UNIVERSITY, JODHPUR-342001 INDIA

Ashok Purohit, H. Ram

Zoology, JNV University, Jodhpur, India

High fat diet caused significant (10 fold) increase in serum cholesterol in rabbits. Administration of Acacia Senegal seed extract (aqueous) at the dose of 500mg/Kg. body weight significantly reduced serum cholesterol (75%), LDL (77%), triglycerides (40%) and phospholipids (30%). The Cholesterol content of aorta was decreased by 50% in seed extract treated group. The HDL to total cholesterol ratio and atherogenic index was significantly decreased in plant treated group suggesting antiatherosclerotic nature of Acacia senegal seed extract. The plant extract feeding brings about a significant regression of atheroma and hindered plaque formation in aorta as compared with hyperlipidemic control group. Thus, this study reflect the antiatherosclerotic property of Acacia senegal seed extract.

Keywords: Antiatherosclerotic effect, Atheroma, Atherogenic index, Acacia senegal.

Memory Stick

INFLUENCING OF RENTGEN-CONTRAST METHODS INVESTIGATIONS OF CORONARY ARTERIES IN PATIENTS WITH ISCHEMIC HEART DISEASE ON THE FUNCTIONAL STATE OF KIDNEY

Nataliya Bardachenko, O. Kuryata

Dnipropetrovsk State Medical Academy, Dnipropetrovsk, Ukraine

Ischemic heart disease (IHD) is a growing health problem in the world. Patients with IHD after angiographic research (AR) have high risk for chronic kidney disease and this problem remains debatable. We aimed to evaluate the kidneys functional state in pts with IHD under the AR of the coronary arteries (CA) and the effectiveness of N-acethylcystein use for the kidneys complications prophylaxis. 594 cardiological department pts´ histories of diseases were analyzed (mean age - 57,68±0,42 years, male - 81% and female - 19%). These pts had angiographic investigations of the CA under recommendation. We picked out 108 pts (18.2%) who had the low glomerulation filtration rate (less than 90 ml/min) before the rentgen-contrast investigations of CA. The levels of creatinine were determined in the blood. The pts were divided into two groups. 54 pts of 1 group had prophylactic infusions of N-acethylcystein before the coronarography (600 mg i/v bolus an hour before the investigation) and 54 pts of 2 group didn't have infusions. The use of N-acethylcystein stipulated for the lower level of the negative creatinine dynamics in 1 group (level of creatinine increased by 4.7% (p>0,05)) compared with the common tactics of coronography investigations without the preliminary infusion of the given medicine in 2 group (level of creatinine increased by 6.6% (p

Memory Stick

HYPERINSULINAEMIA, ENDOTHELIAL DYSFUNCTION, INFLAMMATORY, METABOLIC DISORDERS AND DIABETIC CARDIOMYOPATHY

Victoriya Serhiyenko, O. Serhieynko

Endocrinology, National Medical University, Lviv, Ukraine

The aim of study was to evaluate which vascular, metabolic and inflammatory alterations characterize diabetic cardiomyopathy (DCMP) in patients with type 2 diabetes mellitus.

Material and methods: 38 patients with type 2 DM and DCMP without hypertension (group D); 14 - type 2 DM without hypertension and DCMP (group C); 32 - type 2 DM and hypertension (group E); 15 patients - hypertension and physiological values of OGTT (group B) and 12 age, body mass index matched controls were enrolled (group A). DCMP was diagnosed according to the obtained data using the Doppler echocardiography, 24-hours ECG, 24-hour blood pressure monitoring. Plasma tumor necrosis factor-α (TNF-α), high-sensitive C-reactive protein (hs-CRP) levels were determined by ELISAs; insulin (IRI), C-peptide, leptin, 125I-6-ketoprostaglandin F1α (6-ketoPGF1α), 125I-thromboxane B_2 (TXB2) - radioimmunoassay; lipids - biochemical methods. Homeostatic Model Assessment (HOMA) insulin resistance (HOMA-IR) and HOMA-β-cell function (HOMA-β-CF) indexes were calculated. Statistics: ANOVA.

Results: The most expressed hyperinsulinaemia [IRI (31,23±2,91 mU/ml); increasing of C-peptide (1573,9±119,6 pM/l), leptin (22,03±2,01 mcg/l); plasma inflammatory markers [hs-CRP (4,71±1,26 mg/l); TNF-α (3,6±0,2 ng/l)] and TXB2 concentrations were observed in patients group D. Exposed changes of IRI concentrations were accompanied by HOMA-IR parameters increasing and HOMA-β-CF decreasing. In group D type IV dyslipidaemia was diagnosed. Direct correlations between leptin, triglycerides and low density lipoprotein cholesterol (r=0,77, P=0,001), leptin and HOMA-IR were found.

Conclusions: Patients with type 2 DM and DCMP have strong correlations between hyperinsulinaemia, parameters of insulin resistance, hyperleptinemia, presence of chronic inflammation, lipids disorders, known as components of metabolic syndrome.

Memory Stick

SENSITIVITY AND SPECIFICITY OF CHOLESTEROL MEASUREMENTS IN FAMILIAL HYPERCHOLESTEROLEMIA DIAGNOSIS IN CZECH POPULATION

Tomas Freiberger[1], B. Ravčuková[1], M. Plotěná[1], L. Fajkusová[2]

[1]Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation, [2]Centre of Molecular Biology and Gene Therapy, University Hospital Brno, Brno, Czech Republic

Familial hypercholesterolemia (FH) is a serious inherited lipid disorder diagnosis of which is based particularly on family history and plasma lipid levels measurement. Detection of disease-associated mutations may be helpful to confirm the diagnosis. The aim of this study was to evaluate the sensitivity and specificity of total cholesterol (TC) measurement against mutational analysis in FH diagnostics.

Three groups of patients were analysed:

1. 789 unrelated patients up to 64 years of age with TC levels exceeding the 95th percentile of population, age and sex specific values, and with DNA analysis completed,
2. 664 unrelated patients with disease-associated mutation detected, and
3. 524 relatives of index patients with the mutation detected.

In group 1, a causal mutation in the *LDL receptor* (*LDLR*) and *apolipoprotein B* (*apoB*) gene was detected in 363 (46%) and 205 (26%) individuals, respectively, while no mutation was found in 221 patients (28%). The proportion of patients with no mutation detected was about 20% in age groups under 45 and increased up to 46% in the age group 55-64. Seventy three patients in group 2 (11%) and 190 patients in group 3 (36%) had TC level under 95th percentile.

In conclusion, TC measurement (95th percentile) is sensitive enough for FH diagnostics in general Czech population (89%) but not sufficiently sensitive in relatives of FH patients (64%). The specificity 80% reached in individuals under 45 years declines significantly in older persons.

Supported by grant of MSMT-CR-No. 2B08060. Thank to all physicians participating in the Czech MedPed project.

Memory Stick

BLOOD PRESSURE RESPONSE TO LOW-FAT AND WALNUT PASTE-ENRICHED MEAT VARIES IN VOLUNTEERS WITH PON1 55 AND 192 GENOTYPES

Francisco J Sánchez-Muniz[1], A. Canales[2], S. Bastida[2], M. Nus[2], J. Librelotto[2], M. Guillén[3], D. Corella[3], J. Benedí[4]

[1]Nutrición, Complutense de Madrid, [2]Nutrición, Universidad Complutense de Madrid, Madrid, [3]Department of Preventive Medicine, Universidad de Valencia and CIBER Fisiopatología de la Obesidad y Nutrición, ISCIII, Valencia, [4]Farmacología, Universidad Complutense de Madrid, Madrid, Spain

Introduction: Lipoprotein oxidation and cardiovascular disease risk differ between PON1 192 (Q/R) and 55 (L/M) allele carriers. Moreover, the eicosanoid and antioxidant responses to consumption of walnut paste-enriched meat (WM), considered a functional food, vary considerably between study participants.

Aims: To compare the effects of consuming WM vs. control low-fat meat (CM) on systolic and diastolic blood pressures in 22 volunteers at increased cardiovascular risk carrying different PON1 192/55 polymorphisms.

Material and methods: The study was a 5-wk non-blinded, cross-over, placebo-controlled trial in which the effects of WM and CM on blood pressure were compared in volunteers at high coronary heart disease (CHD) risk.

Results: WM vs. CM consumption did not significantly decrease systolic and/or diastolic blood pressure in all volunteers. However, this nutritional intervention was able to decreased systolic and diastolic blood pressures in LLPON1-55 but not in LM+MMPON1-55 volunteers. The same was observed in diastolic blood pressure in AR+RRPON1-192 volunteers but not QQPON1-192 participants.

Conclusions: The blood pressure response of volunteers at CHD increased risk to WM and CM consumptions is affected by the variants Q192 and/or M55 of the PON1 gene.

Funds for this study were granted by the Spanish Ministerio de Educación y Ciencia, Project AGL 2001-2398-C03 and AGL 2005-07204-C02-01/ALI and Consolider-Ingenio 2010, reference CSD2007-00016.

Memory Stick

AN IMPROVED DEVICE TO REPRODUCE IN VITRO THE PULSATILE CORONARY FLOW CONDITIONS MEASURED IN HUMANS

Kamil Chodzynski[1], K. Zouaoui Boudjeltia[2], G. Coussement[1], J. Lalemand[3], M. Vanhaeverbeek[2]

[1]Fluids-Machines, Faculty of Engineering Mons, Mons, [2]Laboratoire de Médecine Expérimentale (ULB 222 Unit), [3]CHU Charleroi, Montigny-le-Tilleul, Belgium

It is well admitted that the shear stress on the vascular wall plays a key role in the artherogenesis. That was the reason for the development of a device which enabled to reproduce the pulsatile coronary flow conditions measured in humans and more specifically the mechanical stresses to which the vascular tissues are submitted. The in vivo data were collected from two patients and two different coronary arteries with an average diameter of 3.39 mm and 2.52 mm. The simultaneous measured in vivo data (velocity, pressure) were post-processed using Newtonian Womersley's correlations and were compared with non-Newtonian CFD calculations. The results are similar for both calculations. However some differences were noticed in the flow rate. The shear stress calculated by CFD methods varies between -4.606 and 15.124 dyn/cm^2 for the first patient, and from -3.285 to 21.59 dyn/cm^2 for the second one. Those results were used to build a realistic in vitro system to imitate flow rates. The in vitro system consists in two pumps: a peristaltic pump which pumps a mean flow, and a piston pump which provides the pulsations of the flow. A rigid tube was used as a test section to check the behaviour of endothelial cells. The real-time control is based on three digital PID controllers designed in Labview environments. The system reproduces the examined coronary flows with an average error of 1.7908 % for the first patient and 4.0184 % for the second one, and repeatability is +/- 5% and +/- 6% respectively.

Memory Stick

CLINICAL PROFILE OF NEWLY DIAGNOSED DIABETIC PATIENTS IN A RURAL COMMUNITY HEALTH FACILITY

Amudha Kadirvelu[1], S. Hasim[2], Z. Mohd Salleh[3], S. Sadasivan[1], C. Chan[1], S. Yasin[1]

[1]School of Medicine and Health Sciences, Monash University Malaysia, Petaling Jaya, [2]Family Medicine, Klinik Kesihatan Segamat, Johor, [3]Family Medicine, Pejabat Kesihatan Daerah, Segamat, Johor, Segamat, Malaysia

Background: The prevalence of type 2 diabetes mellitus (DM) is increasing globally with huge burden of suffering caused not only by the disease but also its complications. Identifying individuals at risk and early stages of chronic diseases is the first step toward preventive measures.

Objective: The aim of our retrospective study was to explore the clinical characteristics of newly diagnosed diabetic patients in a rural community health clinic.

Methods: The study participants were consecutive newly diagnosed DM patients aged > 20 years presenting to a community health care facility. The data collection was between January-August 2008. The clinical data and biochemical parameters were collected retrospectively by medical record review.

Results: One hundred seventy six patients were included; 11.3% of the study population were < or = 40 years of age; 37.5% were between 40-55 years and 51% were > 55. Of the study subjects, 48.4% were females and 51.6 % were males. Family history of diabetes was present in 22% of the study population. A diagnosis of DM only (fasting plasma glucose levels of > 7.0 mmol/l) was made in 46.6% of the patients while 53.4% were diagnosed with DM and hypertension. Of the subjects with DM and hypertension, 44.4 % were between 40-55 years while 62.8% were > 55 years.

Conclusion: Our study indicates that hypertension is a common co occurrence in newly diagnosed diabetic patients. Increased vigilance, early identification and **i**ntensive management may work towards decreasing the risk of atherosclerotic burden in these patients.

Memory Stick

DESIGNING A STUDY TO EVALUATE THE EFFECT OF APHERESIS IN PATIENTS WITH ELEVATED LIPOPROTEIN(A)

Ursula Kassner

Stoffwechselcentrum Campus Virchow, Charité Universitätsmedizin Berlin, Berlin, Germany

Lipoprotein(a) (Lp(a)) is a risk factor for premature coronary artery disease. Lp(a) levels can not be sufficiently influenced neither by standard hypolipemic diet nor by drug therapy. Currently lipid apheresis is the only option to lower Lp(a) levels in patients with elevated Lp(a) and progressive CVD effectively. In the lipid-clinic at the Charité University hospital Berlin and other german apheresis centres exists longstanding positive experience with this therapeutic regimen.

Lately in Germany lipid apheresis was accepted as a treatment of choice for patients with elevated Lp(a) levels > 60 mg/dl and progressive CVD. At the same time care providers were obliged to conduct a prospective trial to prove the efficacy of lipid apheresis for this indication. Therefore we designed a prospective, randomized, controlled trial to prove the hypothesis that lipid apheresis is a potent therapeutic option.

Memory Stick

CORRELATION STUDY OF MORPHOLOGICAL FEATURES OF SILENT AND VULNERABLE PLAQUES VS CLINICAL PARAMETERS OF PATIENCE AFTER CAROTID ENDARTERECTOMY

Valentina S. Shishkina, S.V. Kashirina, V.N. Sirotkin, E.R. Andreeva, O.P. Ilyinskaya, E.M. Tararak

Cardiocenter, Moscow, Russia

Background: To analyze peculiarities of histological composition of endarterectomy plaques in connection with patients' anamnesis.

Methods: Thirty-six patients were enrolled. Plaques of all patients were divided into 2 groups according to Lovett JK et al, 2005: group I, 26 vulnerable plaques; and group II, 10 silent plaques. All plaques were classified according to AHA classification. All morphometric assessments were blind with clinical parameters to find out correlations between morphological alterations in the artery and there clinical manifestations.

Results: 71% of all carotid plaques were classified as vulnerable and 92% of them were complex (Type VI of AHA). 81% of them were excised from males age 60,3±7,6, 19% from female age 72,3±9,6. Stable plaques were: 40% typeVI, 20% typeIV, 20% typeVa, 10% typeVb and 10% typeVc. 60% of them belong to male, 40% to female age 62,3±9,1 and 70,8±4,3 accordingly. Patients with plaques group I more often had ischemic heart disease ($p< 0,05$), transient ischemic attack (TIA) and/or stroke ($p< 0,05$), had greater degree of stenosis and surface defects, while patients with lesions group II - the disease of gastrointestinal tract ($p< 0,05$). Risks factors, such as smoking, hypertension, angina pectoris and myocardial infarction were more often observed in patients with plaques group I. Two groups of lesions significantly differed ($p< 0,05$) in plaque vascularisation, surface defects, internal thrombus, cholesterol, foam cells, calcium deposits.

Conclusion: Patients with more pronounced cardiovascular events preferentially had plaques group I that should be take into consideration in the strategy of their treatment.

Funding: RFBR-OFI-c-08-04-13753, contract-N/10-ATH-M.

Memory Stick

MARKERS OF SUCCESSFUL THROMBOLYTIC THERAPY DURING THE FIRST WEEK OF ACUTE MYOCARDIAL INFARCTION

Vera Kapetivadze, R. Tabukashvili, K. Tchaava, N. Gegeshidze, Z. Grigorashvili, I. Kapetivadze

Internal Medicine, Tbilisi State Medical University, Tbilisi, Georgia

Objective: The goal of our study was to identify the importance of dispersion of ventricular repolarisation in predicting the course of the acute phase of myocardial infarction because of ischemic localization and initial effectiveness of thrombolytic therapy in patient with Q wave myocardial infarction during the first week after the onset of heart attack. For this purpose, 69 patients were enrolled and divided into three groups. I.patients with acute myocardial infarction on an anterior wall.(54±65 age group) (n=28) II. patients with acute myocardial infarction on the posterior wall (54±65 age group) (n=26) III. control group of healthy individuals without myocardial ischemia. (48±7 age group) (n=15).

Methods: Immediately after hospitalization the patients were given thrombolytic therapy (Aspirin, heparin, stpeptokinaza). Was studied QT interval dispersion (QTd) and variability of QT interval in precordial leads (QTdl).

Result: The study showed that QTd and QTdl could be used for assessment of the risk of malignant arrythmias and effectiveness of throbolytic therapy in patients with acute Q-wave myocardial infarction because of ischemic localization during the first week.

Conclusion: Based on the study results, it can be concluded that the values of QTd and QTdl may be predictors of malignant arrhythmias during the first week of miocardial infarction because of ischemic localization. QTd and QTdl are markers of successful thrombolytic therapy.

Keywords: QT interval dispersion, Thrombolytic therapy.

Memory Stick

TREATMENT OF ATHEROSCLEROSIS PATIENTS WITH TYPE 2 DIABETES BY SOYBEAN FLAVONOID SUPPLEMENTS AND INDAPAMIDE

Vera Kapetivadze, R. Tabukashvili, K. Tchaava, N. Gegeshidze, Z. Grigorashvili, I. Kapetivadze

Internal Medicine, Tbilisi State Medical University, Tbilisi, Georgia

Objective: To study therapeutics action of treatment by soybean flavonoid supplements and Indapamide in atherosclerosis patients with type 2 diabetes.

Methods: Were studied 42 patients atherosclerosis with type 2 diabetes. The patients were divided into 2 groups. GroupI - experimental group -30 patients and group II -control group -12 patients. The patients of groups I were given 200 mg soybean flavonoid and indapamid 2,5 mg a day; the patients of group II were additionally given Indapamide 2,5 mg. a day. The patients were under investigation during 10 weeks. Before and after clinical observations were studied lipid range, the level of glucose in blood.

Results: 10 weeks after clinical observations was obtained decrease of cholesterol. In patients of experimental group: TC by 7%, LDLC by 10%, TG by 9,4%, HDLC increase by 8%. In patients of control group after clinical observations was obtained decrease of cholesterol: TC by 3%, LDLC by 5,6%, TG by 6%, HDLC increase by 7%.The level of glucose in patients of I group before clinical observations was 8,7 mmol/l, after 4,8 mmol/l. in patients of II group the level of glucose before clinical observation was 8,5mmol/l, after 6,2 mmol/l.

Conclusions: Therefore, given of soybean flavonoids coupled with Indapamide in patients atherosclerosis with type 2 diabets are biologically active and give perspective use it in the treatment of the patients.

Memory Stick

THE EFFECT OF PAIN-FREE TREADMILL TRAINING ON LEVELS OF LACTATE DEHYDROGENASE AND CREATINE KINASE IN PATIENTS WITH INTERMITTENT CLAUDICATION

Piotr Mika[1], R. Nowobilski[1,2], R. Januszek[2], H. Plutecka[2], M. Sanak[2], A. Szczeklik[2]

[1]Departament of Clinical Rehabilitation, University School of Physical Education, [2]Departament of Medicine, Jagiellonian University Collegium Medicum, Krakow, Poland

Introduction: Episodes of ischemia-reperfusion occurring during repeated walking may lead to a local and systemic inflammatory response in patients with claudication. Regular treadmill training has been suggested as an effective means for improvement of walking ability and attenuation of inflammation in this group of patients.

Aim: This study aimed to assess the effect of pain-free treadmill training on two [lactate dehydrogenase (LDH) and creatine kinase (CK)] hematological indicators of skeletal-muscle injury.

Method: Fifty patients with peripheral occlusive arterial disease and intermittent claudication were randomly assigned into a treadmill-training program or a control group. The treadmill training consisting of repeated walking exercises to onset of claudication pain was conducted 3 times a week for 3 months. The pain-free and maximal walking times, LDH and CK were assessed in both groups during treadmill test to maximum of claudication pain performed at baseline, after 6 weeks, and after 3 months period of the study.

Results: The significant ($p < 0.05$) increases of LDH and CK levels were observed after treadmill tests in both groups of patients. The LDH and CK levels significantly ($p < 0.05$) decreased by 10.2% and 9.5%, respectively, while pain-free walking time and maximal walking time was prolonged by 115% and 74%, respectively, in the training group after 3 months of treadmill training. No significant changes in the above variables were observed in the controls.

Conclusion: Three-month pain free treadmill training is associated with progressive normalization of LDH and CK in patients with claudication.

Memory Stick

EFFECT OF PLANTS ON THE TREATMENT OF RHEUMATOID ARTHRITIS: A PRAGMATIC RANDOMIZED ETHNOMEDICINAL SURVEY IN FARIDPUR DISTRICT OF BANGLADESH

Md. Ariful Haque Mollik

Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Peoples Integrated Alliance, Dhaka, Bangladesh

Rheumatoid arthritis is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints, which can be disabling and painful. The disease has a worldwide distribution with an estimated prevalence of one percent. This prevalence increases with age and can approach five percent in women over age fifty-five. Since this disorder is also present in Bangladesh, we conducted an ethnomedicinal survey amongst the traditional healers in Faridpur district of Bangladesh to gather information on plants used by the traditional healers to treat this disorder. The rural populations of Bangladesh, mostly often lacking access to modern medicinal facilities rely on traditional healers, who possess in them an incredible knowledge of medicinal properties of plants and often use such plants with success in treating various ailments. Plant samples were collected from the traditional healers and identified at the Bangladesh National Herbarium. The plants mostly used to treat rheumatoid arthritis included *Cinnamomum camphora, Linum usitatissimum*, *Nigella sativa*, *Aconitum napellus*, *Olea europaea*, *Lens esculenta*, *Santalum album*, *Ipomoea aquatica*, *Nicotiana tobacum*, *Alternanthera sessilis*, *Ricinus communis*, *Piper betle*, *Calotropis gigantea*, *Musa sapientum*, *Pongamia pinnata*, *Cyperus rotundus*, *Datura stramonium*, *Polygonum persicaria*, *Withania somnifera*, *Azadirachta indica*, *Curcuma longa*, *Zingiber officinale*, *Cynodon dactylon*, *Lawsonia inermis, Brassica napus*, *Cissus quadranglaris*, *Cocos nucifera*, *Allium sativum*, and *Aphanamixis polystachya*. Since modern medicine is unable to cure this disease but merely addresses the symptoms associated with the disease like pain, the above plants can be of potential importance for further scientific studies leading to complete cure of this debilitating disease.

Memory Stick

THE ENDOTHELIUM AS STARTING FOR COMPLICATED ATHEROSCLEROTIC PLAQUE

I. Zota[1], **Eugen Melnic**[1], A. Munteanu[1], E. Zota[2]

[1]Pathology, [2]Neurology, The State University of Medicine and Pharmacy "N.Testemitanu", Chisinau, Moldova

Introduction: The purpose of this study was to establish the endothelial cells role in the initiation and developing of complicated atherosclerotic plaque. The immune morphological investigations were centered on the study of the levels VCAM-1, eNOS, LDL, IL-1, TNF-α, HSP-60, CRP and CD 40-CD40L.

Material and methods: The studies were made on the basis of human aorta section, coronaries vessels, cerebral artery, collected through early autopsies on the atherosclerosis and associated pathologies deceased patients, as well as through the use of the experimental atherosclerosis models on laboratory animals. The role of VCAM-1, eNOS, LDL, IL-1, TNF-α, HSP-60, CRP, and CD 40-CD40L was investigated through the application of electron microscopy, immune morphologic methods, with use of monoclonal antibodies and radioautographic methods.

Results: Atherosclerosis debut is not conditioned by endothelial tunic injure, but sooner by the endothelial cells functional reorganization which can be traced at the initial stages through CD 40-CD40L studies. The large concentration of NO generated by iNOS contributes to the atherosclerosis progress through a series of mechanisms that include the intensification of LDL oxidation. Endothelial cells, through molecule receptor expressions on the surface of the plasmalema conditions directly the inflamed celss adhearing process and the initiation of immune response, they can express genes that codifies these adjusted molecules of differentiation and leucocytes' function.

Conclusion: The results demonstrate the major role of endoteliocites as an initial phenomenon of atherosclerosis and give us the possibility to determine the strategies regarding new methods of diagnosis elaboration and treatment of atherosclerotic plaque.

Memory Stick

PSYCHOLOGICAL-VEGETATIVE FRUSTRATION AT PATIENTS WITH THE CHRONIC CEREBRAL ISCHEMIA (CCI) AMONG THE POPULATION OF UZBEKISTAN

Nadira Sultankhodjaeva

Neurology, La Sante Clinic, Tashkent, Uzbekistan

CCI is one of the first prevalence of the cerebral atherosclerosis and it is the reason of mortality, disability and losses of social adaptation. The most effective way of early pharmacotherapy and pharmaceutical prevention of the cerebral atherosclerosis is using of means with specific heroprotective effect. The purpose of our research was studying dynamics of cognitive and emotional sphere at patients - invalids with CCI on a background of the cerebral atherosclerosis at stages of rehabilitation, also correction them with preparation-actoveguin. 135 patients-invalids at the age of from 30 till 55 years (middle age 42+3,76) with the diagnosis I and II degree of CCI on a background of the cerebral atherosclerosis were surveyed. The patients were divided into 2 groups. 2-group was taken complex treatment with actoviguin. The estimation of efficiency of treatment was based on results of following researches: clinic-neurological research, psychological-emotional research, in the present work were used test of Spielberg in modification. Screening scale of diagnostics of dementia were researched clinically on the basis of criteria of the American psychiatric association. On a background of treatment at patients (2-group) positive changes from the side psychological-mental spheres was observed. Vegetative sphere of these patients has sharply decreased to normotonic. By encephalography it was marked that tendency of alpha-capacity increased and regress of slow-wave activity index. It confirms about improvement of brain activation and improvement of clinical-neurological semiology. Consequently, application of actoveguin neuroprotective, which metabolic properties affect a subsystem of emotionally affective reaction and it's pathogenetic proved.

Memory Stick

CORONARY ATHEROSCLEROSIS AND METABOLIC SYNDROME: THE WAYS TO TREATMENT OPTIMTSATION

Maryna Gelmedova

Department of Dislipidemia, Institute of Cardiology, Kyiv, Ukraine

Aim: To study the characteristics of atherosclerosis and the condition of coronary vessels in patients with IHD, diabetes metabolic syndrome (MS) to optimize treatment.

Methods: 57 patients with coronary atherosclerosis (verificated by angiography) and with MS components were examined before CABG. After examination, patients were held revascularization. All patients had to make coronarography in 1-year time after operation.

Results: The patients included in clinical groups depending on a quantity of coronary arteries damage: I gr.3 arteries damage, II gr.2 arteries, III gr.1 arteries, IV gr.arteries intact. Were determined: body mass index, blood pressure, lipids, glucose, insulin HOMA. At an HOMA>2.77 defined insulin resistance(IR) and angiography was made. The IR was found in all groups, with the highest value in-group-I and the maximum level of insulin also. The maximum level of cholesterol, triglycerides were in the groupII, but the differences with the group-I was minor. In the analysis of the 24-hour blood pressure monitoring in groupI was found the worst profile-type night-piker. Less dangerous type (non-dipper) was observed in all groups, with a minimum in groupIV. Normal type(dipper) observed in all groups with a minimum in the group-I. Patients who completed all the recommendations 1-year after KABG have a good general condition. Patients who ignored the recommendations have no positive changes in wealth.

Conclusions: The laboratory indices deteriorated in line with the increase in the number of affected vessels. The worst blood pressures profile-type and the most unfavorable prognosis in the group-I. The correct treatment influenced the course of the disease and atherosclerotic damage of vessels.

Memory Stick

EFFECT OF CORNUS MAS L. ON ATHEROSCLEROTIC PLAQUE IN HYPERCHOLESTEROLEMIC RABBITS

Sedigheh Asgary[1], A. Adelnia[2], S. Kazemi[2], M. . Rafieian-Kopaei[3], F. Shamsi[2]

[1]Basic Science, Cardiovascular Research Center, [2]Isfahan Cardiovascular Research Center, Isfahan, [3]Medical Plants Research Center-Shahrecord, Shahrecord, Iran

Atherosclerosis which results from gradual deposition of lipids in medium and large arteries is a leading cause of mortality worldwide. *Cornus mas* L. is a medical plant from Cornaceae family which contains flavonoids and high antioxidants with anti-inflammatory properties. This study was conducted to determine the effect of *Cornus mas* powder on blood lipids and atherosclerosis in rabbits fed with high cholesterol diet. Fifteen male rabbits were randomly divided into three groups [normal diet group, high cholesterol diet (1% cholesterol) and a group which received high-cholesterol diet supplemented with *Cornus mas* powder (1g/Kg body weight every day). Total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), high density lipoprotein (HDL), cholesterol and antioxidant capacity of plasma was determined in rabbits, at the beginning and also end of the study. At the end of the experimental period the aorta was removed for assessment of atherosclerotic plaques.

Cornus mas significantly increased HDL and antioxidant capacity of plasma. It also decreased TC, LDL and TG levels and atherosclerotic lesion in aorta, but the reduction was not statistically significant.

The results showed that consumption of *cornus mas* might be beneficial in hypercholesterolemic patients which might be due to antioxidative and anti-inflammatory properties of *Cornus mas*.

Keyword: Atherosclerosis, Cornus mas, HDL, LDL, Rabbit.

Memory Stick

CORRELATIONS OF BIORHYTHMS IN SERUM LIPIDS AND LIPOPROTEINS WITH ATHEROSCLEROTIC MANIFESTATIONS IN DYSLIPIDEMIC INDIVIDUALS

M. Rodrigues[1], F. Dalpino[2], A. Cassol[2], C. Samogin[2], **Eliana de Faria**[3]

[1]Department of Clinical Pathology, [2]State University of Campinas, [3]Department of Clinical Patology, University of Campinas, Campinas, Brazil

Objective: This study evaluated the effects of dyslipidemia on the occurrence of biological rhythms in serum lipids and lipoproteins and their correlation with atherosclerotic disease in a large Brazilian population sample.

Methods: A retrospective study was carried out to evaluate the lipid profiles from individuals registered at the healthy center, State University of Campinas, during 8 years. The studied population was composed of individuals of both sexes and at all ages totalizing 27,543 participants and 228,748 laboratory exams .Normolipidemic and dyslipidemic individuals, classified according to Brazilian guidelines on Dislipidemias, participated in the study. The frequencies of cardiovascular disease were obtained from the Brazilian data base DATASUS. Statistical analyses were carried out using the SAS program and the temporal analysis used the Cosinor method.

Results: In normolipidemic cases (n=11,892) significant seasonal rhythmicity was observed only in LDL and HDL-cholesterol (respectively $p \leq 0.018$ and $p \leq 0.031$), with higher values in winter and lower in summer. In the dyslipidemic group (n=15,651) significant seasonal rhythmicity was observed in Triglycerides ($p \leq 0.001$), higher in summer and lower in winter, and in Cholesterol ($p \leq 0.021$), LDL-cholesterol ($p \leq 0.001$) and HDL-cholesterol ($p \leq 0.010$) all higher in winter and lower in summer. Positive cross-correlations were observed between the rhythms of LDL-cholesterol and atherosclerosis in normolipidemic individuals and in dyslipidemia of LDL-cholesterol with atherosclerosis, myocardial infarction and vascular diseases.

Conclusion: Dyslipidemia increased the number and amplitude of lipid biorhythms. The correlation between these rhythms with the ones of prevalence of atherosclerosis manifestations indicates the impact of lipid and lipoprotein seasonality on cardiovascular disease in Brazil.

Memory Stick

THE RELATIONSHIP OF STRESS TO PATHOLOGY, PHYSIOLOGY, ANESTHESIA AND ANALGESIA

Lewis Coleman

Anesthesiology, Sierra King Dental Surgery Center, Parlier, CA, USA

This paper proposes a “Stress Mechanism” that continuously repairs and maintains the vertebrate body. It explains how Stressors induce stress. It consists of the Vascular Endothelium, the Autonomic Nervous System, and the enzymatic interaction of blood-borne Factors VII, VIII, IX and X. The Vascular Endothelium is an autonomic nervous gland, ubiquitous throughout the body, that responds to Stressors, including both stressful forces and stressful stimuli. It controls the enzymatic interaction, and thereby determines the location, magnitude, and speed of production of Thrombin, Soluble Fibrin and Insoluble Fibrin, whose multiple effects explain all Stress Mechanism manifestations. Positive Feedback in the Stress Mechanism produces disease. When Stressors subside, Negative Feedback restores Stress Mechanism activity to a “resting state”. Stress Mechanism activity explains Eustress, Distress, Fight-or-Flight, the General Adaptation Syndrome, hemodynamic physiology, Malignancy, Apoptosis, Capillary Hemostasis, Infarction, Anesthesia, Analgesia, Atherosclerosis, Eclampsia, Multi-System Organ Failure (MSOF), Adult Respiratory Distress Syndrome (ARDS), High Altitude Pulmonary Edema (HAPE), Angiodysplasia, Angioneurotic Edema, and the Surgical Stress Syndrome.

Memory Stick

COMPUTER SIMULATION OF PLAQUE FORMATION AND DEVELOPMENT

Nenad Filipovic[1], N. Meunier[2], M. Boynard[2], M. Kojic[3], D. Fotiadis[4]

[1]Faculty of Mechanical Engineering, University of Kragujevac, Kragujevac, Serbia, [2]University Paris Descartes, Paris, France, [3]University of Texas, Houston, TX, USA, [4]University of Ioannina, Ioánnina, Greece

Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. Over the past decade, scientists come to appreciate a prominent role for inflammation in atherosclerosis.

In our computer model we firstly assume the passive penetration of LDL in particular areas of the intima. Once in the intima the LDL is immediately oxidized and when the LDLox exceeds a threshold there is recruitment of monocytes which immediately differentiate into macrophages. Monocytes evolve in macrophages which phagocyte LDLox and evolve in (foam cells) by massive ingestion of LDLox.

The steady and pulsatile flow field with plaque formation and development is analysed in simplified 2D and 3D mild stenosis model. The blood flow is simulated by the three-dimensional Navier-Stokes equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process was solved with three additional reaction-diffusion partial differential equations. The plaque growing was modeled by Stokes equation.

The computed results show velocity profiles, shear stress distribution and LDL distribution in blood lumen. Computed concentration oxidized LDL, macrophages and cytokines indicate that there is a newly formed matter in the intima, especially in the flow separation region of the coronary artery.

A full three-dimensional model of plaque formation and development, coupled with blood flow and LDL concentration in blood, was created. The plaque location and progression in time for a specific patient shows a clear benefit for future vascular modeling and prediction using computer simulation.

Memory Stick

TOTAL ANTIOXIDANT STATUS IN CORONARY ARTERY DISEASE PATIENTS

Aleksandra Vukasinovic, J. Kotur - Stevuljevic, A. Stefanovic, S. Spasic, V. Spasojevic - Kalimanovska, D. Ristic

Institute for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Background and aims: Coronary artery disease (CAD) and atherosclerosis represent leading cause of death in the world. Reduced antioxidant protection is considered to play an important role in pathogenesis of CAD. We investigated if total antioxidant status (TAS) is reduced in patients with CAD and the possibility of using TAS as a confident diagnostic CAD parameter.

Methods: The study comprehended 96 CAD patients and 31 healthy persons as control group. Using method of coronary angiography CAD diagnosis was set. TAS values were measured with ABTS as chromogen. The diagnostic value of TAS was checked by determining other oxidative injury indicators.

Results: Patients had significantly lower TAS values (0,890 mmol/L ±0,4337) compared to control subjects (1,239 mmol/L ±0,3185). Comparing patients without clinical significant stenosis (lower than 50%) to those with clinical significant stenosis at one, two or three blood vessels, the firt ones had significantly higher TAS concentrations ($p < 0,05$). There is no significant difference between patients with clinical significant stenosis. The positive correlations are noticed between TAS and SOD enzymatic activity ($\rho=0,236$; $p=0,027$), while negative ones are obtained with fibrinogen ($\rho= -0,233$; $p=0,027$), HDL diameter ($\rho= -0,444$; $p=0,000$) and superoxide anion ($\rho= -0,198$; $p=0,068$).

Conclusions: Significant correlations with other oxidative stress parameters indicate possibility of using TAS as a diagnostic parameter. Lack of difference in TAS values depending on number of blood vessels with significant stenosis, restricts usage in atherosclerosis progression degree assessment, but suggests need for further research in order to better uderstand the antioxidant protection role in CAD prevention.

Memory Stick

THE VALUE OF D-DIMER IN ACUTE AORTIC DISSECTION: THE EXPERIENCE OF CHINA

Wenlong Li, X. Fan, J. Xu, W. Zhang, X. Wang, K. Sun, R. Hui

Ministry of Education & Sino-German Laboratory for Molecular Medicine, Peking Union Medical College, Beijing, China

Background: Acute aortic dissection(AAD) is a uncommon lethal medical emergency, with a high mortality. D-dimer is a fibrin fragment and always elevates in AAD patient. However, the diagnostic role of D-dimer for AAD remains uncertain. We evaluated the sensitivity, specificity and likelihood ratios of D-dimer in diagnosing AAD in China.

Methods: In this retrospective single-center study, a total of 343 patients with symptoms onset within 24 hours were enrolled. Of them, 127 were diagnosed with AAD by enhanced computed tomography, and 216 non-AAD controls with other diagnoses, including angina(92), acute myocardial infarction(49), pulmonary embolism(8), and other uncertain diagnoses(67). The plasma D-dimer was detected by stago-evolution(France).

Results: The median D-dimer level was significantly higher in AAD patients (4.0ug/mL) than in non-AAD controls(0.39ug/mL)(P=0.000). The median D-dimer level in AAD patients with false lumen(4.40ug/mL) was markedly higher than in AAD cases without false lumen(1.49ug/mL)(P=0.000). Receiver operating characteristic curves analysis showed that D-dimer was predictive to diagnosis AAD, with a sensitivity of 92.9% and specificity of 70.4%. In cutoff level of 0.5ug/mL, the negative likelihood ratio was 0.10, with positive predictive value of 0.64, negative predictive value of 0.94 and positive likelihood ratio of 3.13.

Conclusion: D-dimer levels is a useful indicator to exclude AAD within 24 hours after symptoms onset.

Memory Stick

EVALUATION OF THE EFFECT OF STATINS ON HYPERTENSION CONTROL IN HYPERTENSIVE HYPERCHOLESTEROLEMIC PATIENTS IN CLINICAL PRACTICE

Manuel Morgado[1], S. Morgado[2], I. Verde[1], M. Castelo-Branco[1]

[1]Health Sciences Research Centre, University of Beira Interior, [2]Pharmaceutical Services, Hospital Centre of Cova da Beira, Covilhã, Portugal

Introduction: Some clinical evidence revealed that statins, aside from lowering cholesterol levels, also have an antihypertensive effect.

Objectives: To evaluate the effect of statin therapy on blood pressure (BP) control and levels in hypertensive hypercholesterolemic patients in clinical practice.

Methods: Prospective evaluation of patients attending the hypertension / dyslipidemia medical clinic in the hospital of Cova da Beira Hospital Centre, Covilhã, located in the Central Region of Portugal, from July to November 2009. Hypertensive patients with hypercholesterolemia were randomisedly assigned to the study. Patients were allocated either to the statin group (when taking a statin) or to the control group (when not taking a statin) and BP control and levels of both groups were compared.

Results: A total of 106 hypertensive patients with hypercholesterolemia were randomisedly assigned to the study (78 in the statin group and 28 in the control group). Although there were no significant differences (P >0.05) in both groups concerning mean age, gender, body mass index, antihypertensive pharmacotherapy and serum levels of high-density lipoprotein cholesterol and triglyceride, BP control was higher in the statin group (P = 0.016). Significant lower systolic BP (-7.9 mm Hg, P = 0.031) and diastolic BP (-5.8 mm Hg, P = 0.006) levels were observed in the statin group.

Conclusions: These results indicate that statin therapy significantly improves BP control in hypercholesterolemic hypertensive patients in clinical practice, consistent with results obtained in some randomized clinical trials. These findings might have useful implications for the prevention of cardiovascular events in primary care patients.

Memory Stick

SERUM HSCRP CONCENTRATION ≥ 2.0 MG/L IS ASSOCIATED WITH CAROTID ATHEROSCLEROSIS, IN WOMEN WITH VARYING DEGREES OF GLUCOSE TOLERANCE

Ulrica Prahl[1], J. Wikstrand[2,3], G. Bergström[2,4], B. Fagerberg[1], J. Hulthe[2,3]

[1]Institute of Medicine, Department of Molecular and Clinical Medicine,Wallenberg Laboratory for Cardiovascular and Metabolic Research, Sahlgrenska Academy, University of Got, [2]Wallenberg Laboratory for Cardiovascular Research, and Center for Cardiovascular and Metabolic Research, Gothenburg, [3]AstraZeneca, Mölndal, [4]Physiology Department, Sahlgrenska University Hospital, Gothenburg, Sweden

Background: Rosuvastatin treatment of subjects with high-sensitivity CRP (hsCRP)≥2.0 mg/L has been shown to powerfully reduce cardiovascular risk. We hypothesized that hsCRP ≥2.0 mg/L was associated with increases in carotid intima media thickness (C-IMT), plaque burden and plaque echolucency in the carotid arteries.

Material and methods: A population sample of 64-year-old women (n=635) with varying degrees of glucose tolerance underwent risk factor assessment including measurement of hs-CRP and bilateral ultrasound examinations of the carotid arteries for measurement of IMT, plaque number and area, and plaque echogenicity.

Results: Subjects with hsCRP≥2.0 mg/L had elevated mean and maximum IMT in the carotid bulbs independently of other cardiovascular risk factors compared with those with hsCRP< 2.0 mg/L. There was no difference between the groups in mean plaque area, although the subjects with plaques in the high hsCRP group had larger total plaque area than the subjects with plaque in the low hsCRP group. Plaque echolucency did not differ between the groups.

Conclusion: In this high risk female cohort hsCRP≥2.0 mg/L was accompanied by elevated IMT in the carotid bulbs independently of other cardiovascular risk factors. Total plaque area was greater among women with plaques in the high vs the low hsCRP subgroup. There was no difference in plaque echolucency between the two groups.

Memory Stick

STRONG UP-REGULATION OF *RUNX2* IN DCC-SUSCEPTIBLE *C3H/HE* MICE AFTER FREEZE-THAW-INJURY

Ann-Kathrin Sowa, J. Erdmann, H. Schunkert, Z. Aherrahrou

Department of Medicine II, Universität zu Lübeck, Lübeck, Germany

Introduction: *C3H/He* mice were used as model for Dystrophic Cardiac Calcification (DCC) using freeze-thaw-injury. DCC shares many features with osteogenesis.

Aim: The aim of this study was to analyze the expression-level of transcription-factors involved in osteogenesis and to identify respective target-genes of these for a better understanding of initiation and development of DCC.

Methods: DCC-susceptible *C3H/He* and DCC-resistant *C57BL/6* mice (n=3) were subjected to freeze-thaw-injury to induce calcification. Early at 24 and 72 hours necrotic and healthy myocardium from each mouse were separated. tRNA and cryo-sections from each tissue were prepared for histological analysis and relative-real-time-PCR using the $\Delta\Delta C_t$-method.

Results: Using Calcein-staining calcification-like deposits appear in resistant and susceptible mice 1 day after injury. Calcification progresses in *C3H/He* but not in *C57BL/6* mice 2 days later. Among the tested transcription-factors a 30.26-fold up-regulation of *Runx2* was detected in calcified tissue of *C3H/He*. Low expression was found for *Sox9, Vdr, Nfkb, Msx1, Smad1*, *Smad2* and *Smad4*, none for *Msx2, Twist1* and *Smad3*. Based on this finding we further tested downstream-genes of *Runx2*: *Vdr, Dmp1, Phex, Osterix, Col1a2, IBSP, MMP2, MMP8, MMP9, MMP13, Bglap II, Opn* and *Akp2*. An up-regulation of *Col1a2* (4.45-fold of induction), of *MMP8* (16.55-fold) and of *MMP13* (15.17-fold) was observed.

Conclusion: Infiltrating cells differentiate into osteoblast-like-cells following injury through high expression of *Runx2*, which activates in turn the MMPs-pathway to cleave collagen (type-I, -II, -III). The MMPs/collagen-interactions and their contribution in repair-processes and tissue-remodelling may explain calcification in myocardium of susceptible mice.

Memory Stick

CORONARY ARTERY ECTASIA: CLINICAL AND ANGIOGRAPHICAL EVALUATION

Tolga Aksu, B. Uygur, N. Arat, H. Kısacık

Turkiye Yuksek Ihtisas Hospital, Ankara, Turkey

Objectives: We investigated the prevalence, risk factors and distribution of coronary artery ectasia (CAE) in patients undergoing coronary angiography for suspected coronary artery disease (CAD).

Design: From 2004 to 2005, 12514 consecutive patients were submitted to coronary angiography. Coronary angiograms were independently reviewed by two operators. Distribution of CAE was made according to the classification of Markis and Ramappa.

Results: CAE was detected in 201 patients (1.6%) and was isolated in 30 patients (14.9%) and associated with aterosclerotic coronary artery disease (ACAD) in 169 patients (84.1%). Among CAE patients, there was a marked male preponderance with 78.6%. Baseline features were similar between isolated CAE and ACAD group.

The RCA was most commonly affected by CAE (45.3%). (Cx and LAD, 39.3%, 31.5% respectively) According to the classification of Markis and Ramappa, the majority of patients had type IV and type 4a ectasia. (77.6%, 57.7%respectively).

Markis Class	Ramappa Class	Patient number in Markis	Patients number in Ramappa	Patients percent in Markis	Patients percent in Ramappa
Type 1	Type 1a	7	3	3.5	1.5
	1b		3		1.5
	1c		1		0.5
Type 2	Type 2a	24	15	11.9	7.5
	2b		9		4.5
Type 3	Type 3	14	14	7.0	7.0
Type 4	Type 4a	156	116	77.6	57.7
	4b		37		18.4
	4c		3		1.5

[Markis and Ramappa classification of CAE patients]

Memory Stick

ASSOCIATION OF RS7138803 IN *FAIM2* GENE AND RS7561317 IN *TMEM18* GENE WITH ANTHROPOMETRIC VARIABLES IN A HIGH CARDIOVASCULAR-RISK MEDITERRANEAN POPULATION

Mercedes Sotos-Prieto, O. Portoles, C. Ortega-Azorin, P. Carrasco, M. Guillén, C. Saiz, D. Corella

Genetic and Molecular Epidemiology Unit, University of Valencia, Valencia, Spain

Introduction: Abdominal obesity is associated with higher cardiovascular risk. Several novel obesity genes have emerged from genome-wide association studies (GWAs) as powerful candidates. Moreover, some of these genes have also been linked to the mechanisms regulating food intake. However, the associations are highly dependent of the population characteristics.

Objective: To study the association between some polymorphisms in two relevant novel obesity genes, transmembrane protein18(*TMEM18)* and fas apoptotic inhiubitore molecule2 (*FAIM2)* with anthropometric variables and dietary intake in a high-risk Mediterranean population.

Methods: We included 945 subjects with high cardiovascular risk (average age: 67±6 years) participating in the PREDIMED (PREvención con Dieta MEDiterránea) Study, Valencia, Spain. Anthropometric, clinical, biochemical, genetic and life-style data were obtained. We selected the rs7138803 (*FAIM2)* and the rs7561317 as the most relevant for analysis.

Results: Minor allele frequencies (MAF) for the rs7138803 (*FAIM2)* and rs7561317 (*TMEM18*) were A=0.445 and A=0.188, respectively. We found a statistically significant association between the rs7561317 in the *TMEM18* gene with waist circumference after multivariate adjustment (AA:99.8±12; AG:103.5±11.2; GG:104.8±12 cm; p=0.042). Waist circumference and body weight were also higher in AA homozygous subjects for the rs7138803 in *FAIM2*, without reaching the statistical significance (p=0.255 and p=0.110 respectively). However, we found that AA subjects had higher energy intake than individuals carrying the G allele (GA+GG) after adjustment for sex and age (AA 2107.5±605.3 *vs* GA+GG 2261.3±660.8 Kcal; p=0.045).

Conclusion: The rs7561317 in *TMEM18* was associated with waist circumference and the rs7138803 in the *FAIM2* gene with energy intake in this elderly Mediterranean population.

Memory Stick

HIGHER LEVEL OF IGM ANTI-OXIDIZED LDL ANTIBODIES IS ASSOCIATED WITH A LOWER SEVERITY OF CORONARY ATHEROSCLEROSIS IN PATIENTS ON STATINS

Lourdes Garrido-Sanchez[1], P. Chinchurreta[2], E. Garcia-Fuentes[3], M. Murri[1], F.J. Tinahones[1]

[1]Hospital Clinico Universitario Virgen de la Victoria (Fundacion IMABIS), [2]Hospital Regional Universitario Carlos Haya, [3]Fundacion IMABIS. CIBEROBN, Malaga, Spain

Introduction: Several studies show the association between anti-oxidized LDL antibodies and atherosclerosis. A certain association also seems to exist between the levels of these antibodies and treatment with statins. However, the clinical importance of these autoantibodies is still under discussion.

Objectives: The aim of this study was to determine whether levels of anti-oxidized LDL antibodies are associated with the presence of multivessel coronary artery disease according to whether statins were or were not being taken.

Methods and results: The study included 236 patients who underwent invasive coronary angiography with quantification of coronary atherosclerotic lesions to determine the presence of multivessel coronary artery disease. Measurements were made of IgG and IgM anti-oxidized LDL antibodies. In the group of patients who were taking statins, the percentage of patients with IgM anti-oxidized LDL antibodies below 50^{th} percentile was significantly higher in those with multivessel coronary artery disease (57.4% vs. 37.5%, $P< 0.05$). A logistic regression model in the patients taking statins showed that the variable significantly associated with the presence of multivessel coronary artery disease was the level of IgM anti-oxidized LDL antibodies.

Conclusions: The severity of multivessel coronary artery disease was inversely related with the levels of IgM anti-oxidized LDL antibodies, especially in the patients on statins.

Memory Stick

GENERAL CARDIOVASCULAR RISK IS BETTER ASSOCIATED WITH ARTERIAL STIFFNESS AND CAROTID INTIMA-MEDIA THICKNESS THAN THE FRAMINGHAM RISK IN NON-DIABETIC SUBJECTS

Young-Kwon Kim, Y.-S. Kim, M.-Y. Rhee, M.-M. Lee

Cardiovascular Center, Dongguk University ILsan Hospital, Goyang, Republic of Korea

Background and aims: Arterial stiffness and carotid intima-media thickness (C-IMT) are surrogate markers of vascular damage, independent of conventional cardiovascular disease (CVD) risk factors and risk predicting algorithm such as the Framingham coronary heart disease risk score (F). We sought to evaluate the relation of these surrogate markers of vascular damage with F and general cardiovascular risk score (G) which predicts combined CVD events.

Methods: A total of 1,294 adults (mean age, 50 years; 402 women; 556 hypertensives) were examined. None had clinical atherosclerotic CVD, diabetes, and current medication for hypertension or dyslipidemia. All subjects underwent standard blood tests, heart-femoral (hf) and brachial-ankle (ba) pulse wave velocity (PWV) measurements and carotid ultrasound examination.

Results: Pearson's correlation coefficients for the relationship between G and hfPWV, baPWV and C-IMT were almost 1.5 folds higher than those between F and hfPWV, baPWV and C-IMT. The prevalence of the highest quartile of hfPWV, baPWV or C-IMT was progressively increased according to G or F quartiles (all, $p < 0.001$ for linear trend). Odds ratios of the prevalence of the highest quartile of hfPWV, baPWV or C-IMT were more stiffly increased according to G quartiles compared with F quartiles.

Conclusion: This suggests that G is better associated with arterial stiffness and C-IMT than F. Thus, in clinical practice, G may be more helpful than F to identify high risk subjects in whom further laboratory testing for detection of subclinical vascular disease is potentially needed.

Memory Stick

THE A148G POLYMORPHISM IN THE PARAOXONASE-2 GENE INCREASES THE RISK OF TYPE 2 DIABETIC MELLITUS AND CORONARY HEART DISEASE

Atip Likidlilid[1], T. Peerapatdit[2], N. Hobang[1], D. Tresukosol[2], S. Ratanamaneechat[3]

[1]Biochemistry, [2]Medicine, [3]Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Objective: To investigate the associations of A148G polymorphism in type 2 DM with and without CHD compared with healthy control subjects.

Materials and methods: The study included 149 unrelated control subjects, 155 type 2 DM subjects without history of CHD and 147 type 2 DM with CHD > 50% epicardial stenosis. gDNA was extracted from leukocyte and the variant was determined by PCR-RFLP.

Results: All genotypes of PON2 A148G polymorphism were in consistent with an expected population of Hardy-Weinberg equilibrium. There was a significantly higher frequency of the GG genotype in type 2 DM with and without CHD when compared with control group (p=0.006 and 0.023, respectively) and the G allele frequencies were significantly higher in type 2 DM with and without CHD when compared with control group (p=0.003 and 0.005, respectively). The risks for type 2 DM and CHD were assessed and found that the GG genotype appeared to be the risk of both type 2 DM with and without CHD with odds ratio 4.74 (95% CI:1.56-14.45) p=0.006 and 3.88 (95% CI:1.26-11.99) p=0.023, respectively. While, G allele also appeared to be the risk of both type 2 DM with and without CHD with odds ratio 1.84 (95% CI: 1.22-2.76), p=0.003 and 1.76 (95% CI: 1.18-2.64), p=0.005, respectively.

Conclusions: PON2 A148G polymorphism was associated with type 2 DM with and without CHD. The GG genotype and G allele of this polymorphism were significantly higher in type 2 DM with and without CHD than in healthy group.

Memory Stick

THE ARG399GLN VARIANT IN THE DNA REPAIR ENZYME XRCC1 IS ASSOCIATED WITH INCREASED GENETIC INSTABILITY IN PATIENTS WITH ATHEROSCLEROSIS

Chiara Federici[1], K. Li[2], K. Salpea[2], C. Maubaret[2], S.E. Humphries[2], M.G. Andreassi[1]

[1]Genetics Research Unit, CNR, Institute of Clinical Physiology, Massa, Italy, [2]Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, Royal Free and University College Medical School London, London, UK

Background: DNA repair is a key factor for maintaining genomic stability, including telomere maintenance. Telomere shortening and chromosomal DNA damage in circulating leucocytes have been shown to be associated with coronary artery disease (CAD).

Aim: To test whether common variants in two DNA repair enzymes, XRCC1 Arg399Gln, XRCC3 Thr241Met, are associated with increased levels of chromosomal DNA damage and shorter telomere in patients with CAD.

Methods: The study population comprised 85 patients (57 male; 66.2±10.0 years; mean ±S.D.) with angiographically-proven CAD Micronucleus assay (MN) was used as biomarker of chromosomal damage. Telomere length was assessed with quantitative real-time PCR. PCR-RFLP analysis was performed for each genetic variant.

Results: MN was higher in women (p=0.04) and telomere length was inversely correlated with age (p=0.03). Carriers of at least one variant allele of XRCC1 Arg399Gln had higher levels of MN (19.0±7.1 Arg/Arg vs 22.9±8.6 Arg/Gln Gln/ Gln p=0.03) and shorter telomere length (0.911±0,20 Arg/Arg vs 0.822±0.19 Arg/Gln Gln/Gln p=0.03). No significant effects with the XRCC3 variants were seen.

Conclusions: XRCC1 is involved in repair of single strand DNA breaks, and our results suggest that the reduced function of the 399Gln allele is associated with both chromosomal DNA damage and telomere shortening in coronary artery patients.

Memory Stick

5-LIPOXYGENASE-DERIVED LEUKOTRIENE B_4 MEDIATES HNE-ENHANCED ROS PRODUCTION IN MURINE MACROPHAGES VIA ACTIVATION OF NADPH OXIDASE

Chi Dae Kim, M.R. Yun, H.M. Park, K.W. Seo, S.J. Lee, S.Y. Park, S.S. Bae

Pharmacology, School of Medicine and MRC for Ischemic Tissue Regeneration, Pusan National University, Yangsan, Republic of Korea

4-Hydroxynonenal (HNE) mediates oxidative stress-linked pathological processes, however, its role in the generation of reactive oxygen species (ROS) in macrophages is still unclear. Thus, we investigated the sources and mechanisms of ROS generation in macrophages stimulated with HNE. Exposure of J774A.1 cells to HNE showed an increased production of ROS, which was attenuated by NADPH oxidase inhibitors as well as a 5-lipoxygenase (5-LO) inhibitor, suggesting a potential role for NADPH oxidase and 5-LO in HNE-induced ROS generation. Linked to these results, HNE increased membrane translocation of p47phox promoting NADPH oxidase activity, which was attenuated in peritoneal macrophages from 5-LO-deficient mice as well as in J774A.1 cells treated with a 5-LO inhibitor, MK886 or 5-LO siRNA. In contrast, HNE-enhanced 5-LO activity was not affected by inhibition of NADPH oxidase by either apocynin or p47phox siRNA. In line with an attenuation of HNE-enhanced NADPH oxidase activity by a leukotriene B_4 receptor antagonist, leukotriene B_4 was found to enhance NADPH oxidase activity in macrophages. Taken together, these results suggest that 5-LO plays a critical role in mediating HNE-induced ROS generation in murine macrophages through activation of NADPH oxidase.

Memory Stick

-203A/C POLYMORPHISM OF CHOLESTEROL 7A-HYDROXYLASE (CYP7A1) GENE AFFECTS CYP7A1 ACTIVITY AFTER SHORT-TERM TREATMENT WITH CHOLESTYRAMINE

Jan Kovar[1,2], M. Zimolova[1,2], M. Lenicek[3], L. Vitek[3], M. Jirsa[1], R. Poledne[1,2], J. Pitha[1,2]

[1]Institute for Clinical and Experimental Medicine, [2]Centre of Cardiovascular Research, [3]Institute of Clinical Biochemistry and Laboratory Diagnostics, Charles University, Prague, Czech Republic

The activity of cholesterol-7α-hydroxylase (CYP7A1) - a key enzyme of bile salt synthesis - displays a pronounced circadian variation. The -203A/C polymorphism of CYP7A1 gene affects the response of cholesterolemia to the cholesterol in the diet but it is not clear yet whether this polymorphism affects also diurnal changes in CYP7A1 activity.

To address such a question, we studied diurnal variation of CYP7A1 activity in 16 healthy men - eight homozygous for -203A and eight for -203C allele. The concentration of 7α-hydroxycholest-4-en-3-one (C4) in serum was determined as a marker of CYP7A1 activity. Three experiments were carried out in each of the volunteers: 1) after short-term treatment with cholestyramine (Q), 2) after short-term treatment with chenodeoxycholic acid (CDCA), and 3) without any treatment. The experiments were carried out in at least 3-week intervals and their order was randomized.

No differences in the diurnal variation of CYP7A1 activity evaluated as area under curve of C4 (AUC C4) between -203A and -203C allele carriers were observed in control experiments and after suppression of CYP7A1 activity by CDCA. On the other hand, treatment with Q was associated with more pronounced increase in AUC C4 in -203C allele carriers. In line with these findings, cholesterol concentration decreased 8% after 24 hours of treatment with Q in -203C allele carriers and was not affected in -203A allele carriers.

Our findings suggest that -203A/C polymorphism of CYP7A1 gene affects the short-term response of cholesterolemia to cholestyramine.

Supported by grant 1M0510 from MEYS CR.

Memory Stick

COMPARISON OF A1C AND FASTING GLUCOSE TO DIAGNOSE DIABETES AMONG PEOPLE WITH METABOLIC SYNDROME AND FASTING GLUCOSE >100 MG/DL

Evangelos Liberopoulos, M. Florentin, A. Kei, E. Mountzouri, A. Agouridis, M. Elisaf

Department of Internal Medicine, University of Ioannina Medical School, Ioannina, Greece

Objective: Comparison of glycosylated hemoglobin (A1C) and fasting glucose for the diagnosis of diabetes among people with the metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L).

Methods: This study included 142 consecutive individuals (73 males, mean age 62 years old) with the metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L) but without a self-reported history of diabetes who visited the outpatient lipid and obesity clinic of the University Hospital of Ioannina, Greece from January through September 2009. A1C ≥6.5% and fasting glucose ≥126 mg/dL (7 mmol/L) were used separately to define diabetes.

Results: Overall, 29.5% of patients had both A1C ≥6.5% and fasting glucose ≥126 mg/dL (7 mmol/L), 25.3% had A1C ≥6.5% but fasting glucose < 126 mg/dL (7 mmol/L) and 9.1% had A1C < 6.5% but fasting glucose ≥126 mg/dL (7 mmol/L). More patients reached a diagnosis of diabetes based on the A1C criterion (n=78, 54.9%) compared with the fasting glucose criterion (n=55, 38.7%, p=0.000). Of note, a large proportion of patients (44.8%) with impaired fasting glucose (fasting glucose 100-125 mg/dL; 5.6-6.9 mmol/L) would be classified as diabetics with the A1C criterion.

Conclusions: Implication of the A1C criterion may largely increase the rate of diabetes diagnosis among people with the metabolic syndrome and fasting glucose >100 mg/dL (7 mmol/L).

Memory Stick

ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECTS OF ZIZIPHUS VULGARIS L. IN STREPTOZOCIN-INDUCED DIABETIC ADULT MALE WISTAR RATS

Jalal Solati[1], R. Hajikhani[1], N. Soleimani[2]

[1]Biology Department, [2]Young Researchers Club, Islamic Azad University- Karaj Branch, Karaj, Iran

Herbal medicine and medical plants such as *Ziziphus vulgaris* L. widely used for treatment of disease like diabetes mellitus. We investigated effects of water extracts of *Ziziphus vulgaris* L. fruit on serum glucose, triglycerides, LDL-cholesterol, HDL-cholesterol and activities of aminotransferase enzymes in streptozocin-induced diabetic adult male rats. Continuous supplementation of this water extract by gavage at dose of 0.25, 0.5,1, 1.5 and 2 g/kg at 0.5ml distilled water in diabetic rats result a significant diminution of fasting blood glucose, LDL-cholesterol and triglycerides level after 14 days. The levels of HDL-cholesterol and Insulin and activities of serum aminotransaminase enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were changed not significantly in the extract supplemented group in respect to control group.

In conclusion, the present study demonstrates that Ziziphus vulgaris possesses significant Antihyperglycemic and antihyperlipidemic properties thus suggesting its beneficial effect in the treatment of diabetes mellitus.

Memory Stick

DIFFERENTIAL EFFECT OF SARTANS WITH VARIOUS PPARΓ ACTIVATING CAPACITY COMBINED WITH ROSUVASTATIN ON GLUCOSE METABOLISM INDICES

C. Rizos[1], H. Milionis[1], M. Kostapanos[1], M. Florentin[1], C. Kostara[2], M. Elisaf[1], **Evangelos Liberopoulos**[1]

[1]Department of Internal Medicine, University of Ioannina Medical School, [2]Laboratory of Clinical Chemistry, School of Medicine, University of Ioannina, Ioannina, Greece

Introduction: Statins have been linked with new-onset diabetes. Some sartans activate peroxisome proliferator activated receptor-γ (PPARγ), favorably affecting glucose and lipid metabolism. Among sartans telmisartan is the most potent PPARγ activator followed by irbesartan, while olmesartan does not hold such capacity.

Objective: We assessed the effects of combination of sartans with different PPARγ activating capacity with rosuvastatin on glucose homeostasis in patients with mixed dyslipidemia, stage 1 hypertension and prediabetes.

Methods: Patients were randomized to rosuvastatin (10 mg/day) plus telmisartan 80 mg/day (RT group, n=52), irbesartan 300 mg/day (RI group, n=48) or olmesartan 20 mg/day (RO group, n=51). Changes in glucose metabolism indices (primary endpoint), lipid profile, blood pressure and high sensitivity C-reactive protein (hsCRP) were determined.

Results: At 6 months, the HOmeostasis Model Assessment Insulin Resistance index improved in the RT group (-29%) compared with RI (+16%; $p< 0.01$ vs RT) and RO group (+14%; $p< 0.05$ vs RT) ($p< 0.05$ for all vs baseline). While fasting plasma glucose and glycated hemoglobin remained unchanged in all groups, insulin levels decreased in the RT (-21%) but not in RI (+12%; $p< 0.01$ vs RT) and RO groups (+8%; $p< 0.05$ vs RT) ($p< 0.05$ for all vs baseline). A difference in hsCRP levels was also noted (-44% in the RT vs -12% in RI vs -20% in the RO group; $p< 0.05$ for RT vs both baseline and RI/RO groups). Blood pressure and lipid changes were similar in all groups.

Conclusion: Tratment with telmisartan plus rosuvastatin improved glucose metabolism indices.

Memory Stick

EFFECT OF STRESS ON CARDIOVASCULAR ACTIVITY AND SERUM CHOLESTEROL IN NORMOTENSIVE AND HYPERTENSIVE RATS

Yana Kuznetzova, O. Semyachkina-Glushkovskaya, V. Berdnikova, I. Semyachkin-Glushkovskij

Biology Department, Saratov State University, Saratov, Russia

Aims: We examined the effect of immobilization stress (60 min) on cardiovascular stress-responses and serum cholesterol levels in normotensive and hypertensive rats.

Methods: The experiments were carried out on mongrel male rats. To induce high levels of blood pressure (BP) rats were been clipped at the renal artery with a silver clip (Goldblatt hypertension, 2K,1C). Rats were instrumented with polyethylene catheters in artery for measuring of mean arterial pressure (MAP) and heart rate (HR). The hemodynamic parameters were measured using PowerLab system for direct recorder of blood pressure signals. Serum total cholesterol was determined with automated methods by a spectrophotometric assay.

Results: Results showed that ischemia of kidney was accompanied by development of hypertension. Stress induced greater increase in HR and more prolongation of increase in MAP in hypertensive males vs. normotensive males. The basal cholesterol levels were significant lower in hypertensive males than in normotensive males. Stress resulted to increase in total cholesterol concentration in both groups of rats. Interestingly notice that stressed levels of cholesterol were higher in hypertensive males vs. normotensive males.

Conclusion: Thus our data suggest that development of renal hypertension is characterized decreased basal serum cholesterol. But despite this fact in hypertensive rats vs. normotensive group stress induces more pronounced increase in cardiovascular stress-reactivity which accompanied by more significant increase in serum cholesterol levels. Research Project GK-144P.

Memory Stick

FAR-INFRARED ATTENUATES CYCLIC STRAIN-INDUCED ENDOTHELIN-1 EXPRESSION VIA PI3K/AKT SIGNALING PATHWAY IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Tzu-Hurng Cheng[1], C.-H. Chen[2], Y.-H. Hsu[2], J.-C. Liu[2], J.-J. Chen[3]

[1]Department of Biological Science and Technology, China Medical University, Taichung, [2]Taipei Medical University-Shuang Ho Hospital, [3]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan R.O.C.

Introduction: Recent studies indicate that far infrared (FIR) therapy exerts beneficial effects in the cardiovascular system. However, the molecular mechanism of the biological effect of FIR is still unknown.

Aims: The aims of this study were to test the hypothesis that FIR may alter strain-induced endothelin-1 (ET-1) secretion, and to identify the putative underlying signaling pathways in human umbilical vein endothelial cells (HUVECs).

Methods: Cultured HUVECs were treated with cyclic strain under FIR exposure, ET-1 expression was examined. Activation of extracellular signal-regulated protein kinase (ERK) and endothelial nitric oxide synthase (eNOS) were assessed by Western blot analysis.

Results: In FIR exposure experiments, the strain-induced ET-1 expression was significantly reduced after FIR exposure. This inhibitory effect peaked with FIR exposure at an effective intensity of 0.13 mW/cm^2 for 30 min, and was only slightly correlated with a thermal effect. The strain-induced phosphorylation of ERK in HUVECs was also inhibited by FIR exposure. On the contrary, FIR exposure induced the phosphorylation of eNOS and nitric oxide (NO) generation in HUVECs. The NOS inhibitor N^G-nitro-l-arginine methylester (L-NAME) and the siRNA transfection for eNOS partially attenuated the inhibitory effects of FIR on strain-induced ET-1 expression and ERK phosphorylation. In addition, wortmannin, a specific inhibitor of phosphoinositide 3-kinases (PI3K), abolished the FIR-induced phosphorylation of eNOS in HUVECs. FIR was also found to up-regulate PI3K expression and induce the phosphorylation of Akt.

Conclusion: These data suggest that FIR attenuates strain-induced ET-1 expression via the PI3K/Akt pathway and NO generation in HUVECs.

Memory Stick

IMMUNE MODULATION BY KLF2 TO IMPROVE COLLATERAL ARTERY DEVELOPMENT

Cansu Yıldırım, T.C.T.M. van der Pouw-Kraan, J.M. Baggen, R.D. Fontijn, A.J.G. Horrevoets

Molecular Cell Biology and Immunology, VU Medical Center, Amsterdam, The Netherlands

Statins, known for their protective role in atherosclerosis, have a positive effect on coronary collateral development, induce expression of the atheroprotective transcription factor KLF2, and inhibit IFN-β production in a murine macrophage cell line.

Recently, we have shown that LPS-activated monocytes from coronary atherosclerotic patients with poorly developed collaterals show an exaggerated interferon-beta (IFN-β) response. Additionally, in a murine hind limb model of arteriogenesis, IFN-β was shown to be a potent inhibitor of collateral formation. Therefore, we hypothesized that inhibition of IFN-β production and/or signalling may serve as a novel approach to stimulate collateral artery growth.

In the present study, we investigated whether KLF2 and statins are capable to inhibit IFN-β production/signalling in human primary monocyte-derived macrophages, and whether KLF2 is the effector molecule of statins in this inhibition.

Expression of KLF2 was studied after transduction with a CD68-promoter driven lentiviral-KLF2 vehicle or statin-treatment. Cells were also transfected with KLF2- or control-siRNAs prior to statin-treatment. Finally, cells were stimulated with LPS and/or IFN-β, and real-time RT-PCR was performed to measure the effect on IFN-β and IFN-β response genes.

Our results show that KLF2 and statins interfere with IFN-β production and signaling in human macrophages. We demonstrate that KLF2 is necessary for the statin-mediated inhibition of IFN-β and IFN-β response genes, implicating that pharmacological enhancement of KLF2 would potentially promote collateral artery development.

Memory Stick

PARAMETERS AFFECTING PROGNOSIS OF PATIENTS WITH ST ELEVATION MYOCARDIAL INFARCTION. AN AMI REGISTRY

Alexandros Gotsis, I.-A. Kerasidou, A. Gagalis, A. Risggits, D. Theodoridis, A. Koutsogianni, L. Borghi, P. Bozia, M. Benechoutsou, A. Panagiotidou

Cardiology, General Hospital of Komotini, Komotiní, Greece

Objectives: The aim of this registry was to study the epidemiological and clinical characteristics of AMI (according AHA-WHF-ESC statement), in order to both prevent AMI and treat patients effectively. The aim of this study is to identify the parameters that affect positively prognosis of patients hospitalized with STEMI.

Methods: We studied 724 patients (506 men, aged 66.6 and 216 women, aged 73.3 years old) hospitalized in our department from January 2005 up to December 2007 with discharge diagnosis of AMI. Among them 349 patients (242 men, aged 64.5 and 107 women aged 71.9 years old) were admitted with STEMI. A detailed medical history was taken emphasizing to risk factors and previous cardiovascular events. Statistical analysis was performed by using SPSS 10.0. The methods used were x^2, Mann-Whitney test and Binary Logistic Regression.

Results: Older patients ($p< 0.001$), women ($p=0.003$), diabetic patients ($p=0.025$) and patients who did not receive GP IIb-IIIa inhibitors ($p=0.005$) had increased mortality. Based to the results mentioned above, there was an effort to create an algorithm of mortality prediction for patients with STEMI by using Binary Logistic Regression. As a result, male sex ($p=0.003$, OP=3.023) and GP IIb-IIIa inhibitors' administration ($p=0.015$, OP=0.219) appear to be independent factors of positive prognosis for patients with STEMI.

Conclusions: Older patients, women, diabetics and patients who did not receive GP IIb-IIIa inhibitors hospitalized with STEMI had poor prognosis. According to our algorithm of mortality prediction, men and patients who received GP IIb-IIIa inhibitors (independently of fibrinolytic therapy administration) have positive prognosis.

Memory Stick

LIPID AND PLEOTROPIC EFFECTS OF LONG-TERM ATORVASTATIN THERAPY IN HYPERTENSIVE DIABETIC AND NON-DIABETIC PATIENTS WITH CORONARY ARTERY DISEASE

Olga Koshelskaya, A. Sushkova, T. Suslova, R. Karpov

Institute of Cardiology, Tomsk, Russia

Objective: This open randomized study was aimed at determine of effects of six months-long atorvastatin (A) treatment on plasma levels of lipids, proinflammatory factors and on arterial stiffness in pts with high cardiovascular risk.

Methods: The 36 hypertensive pts with CAD and dyslipidemia divided into two groups: diabetic pts (Gr.1, n=19) and pts without diabetes (Gr.2, n=17) took part in this study. In all pts, A-dose was increased from 10mg up to 40mg/day if the target level of LDL was not achieved. There were estimated: plasma levels of the lipids, HbA_1, hsCRP, TNF-α, IL-1β, IL-6, IL-10 and of leptin. A carotid sonography was performed and artery stiffness was calculated by the β stiffness index.

Results: After the 6 months of therapy mean doses of A were 16,3mg/day and 16,4mg/day in Gr.1 and Gr.2, respectively. Mean values of LDL-C levels were decreased to the same extent in both groups of pts, whereas ApoB/$ApoA_1$ levels were decreased only in Gr.2. In both groups, we found the significant decrease in median values of hsCRP, TNF-α and IL-1β, whereas the IL-6 and leptin plasma levels were decreased only in Gr.1. Beta stiffness index being unchanged in both groups and was increased in those diabetic pts who had inadequate BP-control.

Conclusions: These data showed the same degree of hypolipidemic and antiinflammatory effects of atorvastatin in diabetic and non-diabetic pts but different dynamic of ApoB/$ApoA_1$ and of leptin plasma levels. In diabetic pts, atorvastatin effect on arterial stiffness is modulated by the degree of BP-control.

Memory Stick

CORONARY ARTERY ECTASIA: CLINICAL AND ANGIOGRAPHICAL EVALUATION

Tolga Aksu, B. Uygur, N. Arat, H. Kısacık

Turkiye Yuksek Ihtisas Hospital, Ankara, Turkey

Objectives: We investigated the prevalence, risk factors and distribution of coronary artery ectasia (CAE) in patients undergoing coronary angiography for suspected coronary artery disease (CAD).

Design: From 2004 to 2005, 12514 consecutive patients were submitted to coronary angiography. Coronary angiograms were independently reviewed by two operators. Distribution of CAE was made according to the classification of Markis and Ramappa.

Results: CAE was detected in 201 patients (1.6%) and was isolated in 30 patients (14.9%) and associated with aterosclerotic coronary artery disease (ACAD) in 169 patients (84.1%). Among CAE patients, there was a marked male preponderance with 78.6% (158 males, 43 females). Baseline features were similar between isolated CAE and ACAD group. Admission complaints were similar between groups except for ACS which was higher in ACAD group. (p=0.016).

A detailed angiographic description of the coronary lesions observed in the 201 patients is reported. The RCA was most commonly affected by CAE (45.3%). (Cx and LAD, 39.3%, 31.5% respectively) According to the classification of Markis, the majority of patients (77.6%) had type IV ectasia. (Table 1) According to Ramappa classification, Tip IVa was the most common class (57.7%) (Table 1).

Markis Class	Ramappa class	Patients Number Markis	Patients Number Ramappa	Patients Percent (%) Markis	Patients Percent (%) Ramappa
Type 1	Type 1a	7	3	3.5	1.5
	Type 1b		3		1.5
	Type 1c		1		0.5
Type 2	Type 2a	24	15	11.9	7.5
	Type 2b		9		4.5
Type 3	Type 3	14	14	7.0	7.0
Type 4	Type 4a	156	116	77.6	57.7
	Type 4b		37		18.4
	Type 4c		3		1.5

[Markis and Ramappa classification of CAE patients]

Conclusion: The clinical course and treatment of CAE mainly depends on its coexistence with CAD. When coexisting with CAD, the prognosis and treatment of CAE are the same as for CAD alone. For clarifying the mechanism underlying CAE, additional clinical, histopathological and pathophysiological investigations are required.

Memory Stick

DISTRIBUTION AND SEVERITY OF ATHEROSCLEROSIS IN RENAL ARTERY: AN AUTOPSY STUDY

Anjali Aggarwal[1], K. Kapur[2], B. Singh[2]

[1]Post Graduate Institute of Medical Education and Research, [2]GMCH, Chandigarh, India

Background and aims: Atherosclerosis has been described as the most common cause of renal artery stenosis. The purpose of this autopsy study was to assess the frequency, distribution and severity of atherosclerotic changes in renal artery in different age groups in normal population.

Methods: Ninety renal arteries from 45 noncatastrophic persons above 30 years of age were obtained at autopsy. Fifty four renal arteries were studied grossly after Sudan IV staining for extent and severity of fatty deposits in terms of Atherosclerotic index (AI). Another 36 renal arteries were studied microscopically for changes in different layers and at different sites of artery and luminal narrowing, if any with advancing age.

Results: In grossly stained specimens, incidence and AI which is the marker of extent and severity of lesions were found to increase gradually with advancement of age. Increased incidence of atherosclerotic changes with better nutritional status was recorded. In microscopically studied specimens, intimal thickness which is a marker of disease also showed upward rise with advancing age. Renal artery stenosis was prevalent in 13.8% cases. Lesions were most commonly detected at renal ostium and proximal segment.

Conclusions: Fatty changes appear with advancing age. Advanced types of changes including fibrous plaques, calcification and ulceration were noticed first in fifth decade. The changes were usually bilateral. Proximal segment was the most affected part. Four cases had less than 50% and one case had 70% luminal narrowing. The changes were only moderately severe in most of the cases.

Memory Stick

EFFECTS OF ANTIVIRAL TREATMENT ON SERUM LEPTIN LEVELS AND LIPID PROFILE IN PATIENTS WITH CHRONIC HEPATITIS C

Tatjana Stojakovic[1], C. Putz-Bankuti[2], H. Scharnagl[1], B. Bauer[3], H. Pock[2], M. Trauner[2], R.E. Stauber[2]

[1]Clinical Institute of Medical and Chemical Laboratory Diagnostics, [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, [3]Department of Internal Medicine, Landeskrankenhaus Hoergas, Gratwein, Austria

Introduction: Elevated triglyceride levels during interferon treatment of chronic hepatitis C (CHC) have been repeatedly demonstrated but the mechanism for this change is still unknown. The aims of our prospective study were to investigate the effects of antiviral treatment with peginterferon (pegIFN) plus ribavirin on serum adipokine levels, insulin resistance and lipid status in patients with CHC.

Methods: Fifty-three treatment-naïve patients (35 males and 18 females) were treated with pegIFN alpha 2a or alpha 2b plus ribavirin. We measured levels of leptin, adiponectin, body mass index (BMI), insulin resistance, lipid profile and HCV RNA at baseline and during antiviral treatment. Continuous clinical and biochemical variables were analysed by repeated measures analysis of variance.

Results: After 12 weeks of treatment leptin concentrations were significantly reduced (-21%, *P*=0.001) and leptin to adiponectin ratio was significantly increased (+53%, *P*=0.016) compared to baseline, the adiponectin concentration remained unchanged. BMI was lowered by 3% (*P*=0.002). Total cholesterol, LDL cholesterol and HDL cholesterol were significantly reduced (-15%, -19%, and -25%, respectively; *P*< 0.001). Triglyceride levels significantly increased (+45%, *P*< 0.001) whereas HOMA IR was not affected. Analysis according to virological treatment response revealed no differences between responders and non-responders.

Conclusions: This study revealed a significant reduction of leptin levels and an increase of the leptin to adiponectin ratio during antiviral treatment with pegIFN plus ribavirin. The relation of these adipokine changes to the observed alterations of the serum lipid profile and to virological response requires further research.

Memory Stick

RENAL FUNCTION AND ATHEROSCLEROTIC PLAQUE COMPOSITION

Guus van Lammeren[1], J.-P. De Vries[2], P. Blankestijn[1], W. Peeters[1], D. De Kleijn[1], A. Schoneveld[1], A. Vink[1], F. Moll[1], G. Pasterkamp[1]

[1]University Medical Center Utrecht, Utrecht, [2]St Antonius Hospital Nieuwegein, Nieuwegein, The Netherlands

Background and aims: Impaired function of the renal system is associated with a higher prevalence of systemic atherosclerotic disease and an increased inflammatory response. The objective of the current study was to examine the relation between renal function and atherosclerotic plaque composition. We hypothesized that impairment of renal function is associated with an inflammatory phenotype of atherosclerotic plaques.

Methods: Atherosclerotic plaques were harvested from 942 consecutive patients, who underwent carotid endarterectomy. We examined the presence of the following immuno-histological characteristics: calcifications, macrophages, smooth muscle cells, collagen, percentage of fat and presence of thrombus. In addition, expressions of a total of 27 cytokines, chemokines and proteases were assessed in a subgroup of 497 randomly selected carotid plaques.

Results: Overall, histological plaque characteristics hardly differed between patients with normal renal function, early or moderate renal failure. Heavy calcifications were observed in 28%(64/227), 27% (116/422) and 36% (98/269) of patients with glomerular filtration rate >90, 60-89; and < 60 ml/min/1.73m^2 respectively (p 0.016). In addition, rantes (CCL5) showed a weak correlation with renal function (r= -0.095; p=0.026). No other histological characteristics or pro-inflammatory proteins were associated with impairment of renal function.

Conclusion: Impaired renal function is associated with an increase of heavy atherosclerotic plaque calcifications. No relation between renal function and local atherosclerotic plaque inflammation could be observed, which suggests that acceleration of local plaque inflammation is not the primary underlying mechanism that explains the progression of cardiovascular disease and poor clinical outcome in patients with renal insufficiency.

Memory Stick

CYSTATIN C AND SUBCLINICAL PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH TYPE 2 DIABETES

José Mancera[1], J. Rioja[2], M.-J. Ariza[2], F. Paniagua[1], L. Ginel-Mendoza[1], M.-Á. Sánchez Chaparro[2], P. González-Santos[2], P. Valdivielso[2]

[1]Servicio Andaluz de Salud. Centro de Salud 'Cuidad Jardin', [2]Departamento de Medicina. Facultad de Medicina, Universidad de Málaga, Málaga, Spain

Aim: To evaluate the relationship between common markers of renal dysfunction and the presence of subclinical peripheral arterial disease (PAD) in patients with type 2 diabetes.

Patients and methods: 436 patients with type 2 diabetes (T2D), aged 50 to 80 years-old were recruited from a single primary care centre. Age, prevalent diseases and medication, anthropometrical data, biochemical markers from fasting blood samples and ankle-brachial index (ABI) were assessed from all the participants. The presence of PAD was considered when ABI < 0,9. We measured serum creatinine and cystatin C as well as urinary albumin/creatinine ratio (ACR). We estimated GFR by the Cockroft-Gault index, and by the Cystatin C index. Patients were categorized as albuminuric when ACR > 30 mg/g. KDQI categories were used to stratify patients according to eGFR. The prevalence (%) of PAD was assessed for each marker of renal dysfunction in intervals of clinical interest using the chi-square statistic.

Results: PAD was found in 126 subjects (29%). Among them, 1 had a serum creatinine > 2 mg/dL, 39 (32%) had an eGFR (Cockroft-Gault) < 90 ml/mt, and 24 (19%) had ACR > 30 mg/g; prevalences were not different compared to controls ($p>0.05$). However, prevalence of abnormal Cystatin C eGFR was signifcantly higher in subjects with PAD compared to those without PAD, specially among those in the subgroup 30-60 ml/mt (14% vs 7%, $p < 0.05$).

Conclusion: Cystatin C eGFR is a better marker for occult PAD than other markers of kidney damage.

Memory Stick

ATHEROTROMBOGENIC AND ELECTRICAL INSTABILITY MARKERS IN CHD PATIENTS UNDER FAT LOADING

Nana Nikolaishvili, N. Kakauridzei, N. Kipshidze

Academician Nodar Kipshidze National Center of Therapy, Tbilisi, Georgia

The aim of our research are to reveal some electrocardiographical predictive markers (myocardium electrical instability parameters) in CHD aged patients; interaction between lipids and thrombogenic risk factor -Fibrinogen (Fb) under fat rich food loads.

Materials and methods: I group - 75 patients with CHD (60.9±8.9 years), II group - 45 healthy persons (58.2 ±7.4 years). Patients were loaded by ,,atherogenic" food. Blood was taken after 13 hours fasting and 3 hours after nutrition load. Lipid profile was performed by enzyme method and Fb - by Bio Fibrin. We studied: QT and JT interval dispersion and its corrected interval dispersion by standard formulas to reveal heart electric instability.

Results: After nutritional loading in I group was revealed increased level of TC, LDLC, TG, Fb ($p< 0.0001$) and decreased level of HDLC ($p< 0.003$), but in control group only TC and TG ($p< 0.0004$) increased significantly. There were confident differences of TC, LDLC, TG ($p< 0.0001$, $p< 0.03$, $p< 0.0001$ respectively) between I and II groups before loading. Fb confidently increased only after loading ($p< 0.01$) in CHD patients. In case of lipemia, in I group there was determined electrical instability which was revealed by confident changes of QTd, QTcd, JTd, JTcd.

Conclusions: Saturated fatty acids rich food provoke the electrical instability (ionic disturbance), revealed by evident increases of QTd, JTd . The fat load may be used as the test for revealing of sudden cardial death risk in CHD aged population CHD.

Memory Stick

OVEREXPRESSION OF AN ABCC6-ENCODING PROTEIN VARIANT FROM C3H/HE ENHANCES CALCIFICATION IN MESENCHYMAL STEM CELLS

Ann-Kathrin Sowa, J. Erdmann, C.L. Doehring, S. Wrobel, H. Schunkert, Z. Aherrahrou

Department of Medicine II, Universität zu Lübeck, Lübeck, Germany

Introduction: Abcc6 belongs to a gene family encoding for ABC-transporters. In mice, Abcc6 was found to predispose C3H/He strains to dystrophic cardiac calcification (DCC). The sequence of Abcc6 varies in seven base pairs leading to amino acids exchanges between DCC-resistant C57BL/6- and DCC-susceptible C3H/He-mice.

Aim: The aim of this study is to examine the role of these protein-variants in calcification in vitro.

Methods: Both variants of cDNA were cloned and inserted in a pSG5 expression vector (pSG5-Abcc6-C3H/He, pSG5-Abcc6-C57BL/6). We established a calcifying cell-culture-model using the mesenchymal stem-cell-line C3H10T1/2 and adding inorganic phosphate to the media. Cells were transfected with empty pSG5 vector (pSG5), pSG5-Abcc6-C3H/He and pSG5-Abcc6-C57BL/6 and analyzed 3, 7 and 21 days after induction of calcification by Alizarin-Red-S-staining and quantification using Randox Ca Kit.

Results: The time-course-analysis revealed no calcification after 3, an initiation of calcification after 7 and a strong calcification after 21 days. The mean values of calcium-deposits were measured to be 9.42 µmol in pSG5, 13.76 µmol in pSG5-Abcc6-C3H/He and 9.13 µmol in pSG5-Abcc6-C57BL/6-transfected cells after 7 days (n=3x3). Interestingly, a significant increase in calcium-deposits was found in pSG5-Abcc6-C3H/He- transfected cells compared to pSG5-transfected (13.75 vs. 9.42; p=0.0063). A non significant decrease in calcium-deposits was observed in pSG5-Abcc6-C57BL/6-transfected cells compared to pSG5 (9.13 µmol vs. 9.42 µmol; p=0.7321).

Conclusion: Using cell-culture-model, we functionally demonstrate for the first time the effect of the amino-acid-substitutions found in the C3H/He-Abcc6 on calcification in vitro.

Memory Stick

VITAMIN E ISOMERS TOCOTRIENOLS CONFER RESISTANCE TO ISCHEMIC INJURY IN THE HYPERCHOLESTEROLEMIC RABBIT HEARTS

Dipak K Das

Cardiovascular Research Center, University of Connecticut Medical Center, Farmington, CT, USA

Most clinical trials with α-tocopherol could not alter the levels of cholesterol and thus, have been deemed unsuccessful. Recently, tocotrienols, isomers of vitamin E have been found to lower LDL levels. To explore the mechanisms of action, rabbits were kept on high cholesterol diet for 60 days and supplemented with tocotrienol α, tocotrienol δ, or tocotrienol γ for the last 30 days. The serum cholesterol levels were 24.4 mmol/L [tocotrienol α], 34.9 mmol/L [tocotrienol δ], 19.8 mmol/L [tocotrienol γ] vs.39.7 mmol/L [control]. Left ventricular function exhibited significantly improved recovery with tocotrienol γ and tocotrienol α, but not with tocotrienol δ. The myocardial infarct size showed a similar pattern: 33% [tocotrienol α], 23% tocotrienol γ], and 47% [tocotrienol δ] To examine the molecular mechanisms , gene expression profile was determined using Atlas 1.2 and 1.2II followed by determination of gene profiles using PedQuest 8.3 software. Based on the genomics profiles, the following cholesterol-related proteins were examined: TGF β [cholesterol suppresses TGFβ], ET-1 [increased by hypercholesterolemia] SPOT 14 [linked with hypercholesterolemia] and matrix metalloproteinase [MMP] 2 and MMP9 [cholesterol regulates MMP2 and MMP9 expression] in the heart. Consistent with the results of cardioprotective effects of tocotrienol α and γ, these two isomers reduced ET-1, decreased MMP2 and MM9 expression, increased TGFβ expression and reduced SPOT 14 expression, while tocotrienol δ had no effects. The results demonstrate that tocotrienols α and γ render the hypercholesterolemic hearts resistant to ischemia by lowering MMP2, MMP9, ET-1 and SPOT 14 and upregulating TGFβ.

Memory Stick

INTERRELATIONSHIP OF CORONARY ARTERY CALCIFICATION, INFLAMMATION AND GLOBAL LEFT VENTRICULAR FUNCTION IN PATIENTS WITH CHRONIC CORONARY ARTERY DISEASE

Ivana Burazor[1], M. Burazor[2], M. Krstic[1], Z. Radovanovic[3], D. Ilic[3], V. Atanaskovic[1], M. Lazovic[1], J. Djordjevic[1], V. Stojanovic[1]

[1]Cardiology, Clinical Center in Nis, [2]Cardiology, University Clinical Center in Nis, [3]Institute for Radiology, Clinical Center, Nis, Serbia

Coronary calcium assessment is being increasingly used in clinical practice. We aimed to evaluate the possible relationship between coronary calcification, inflammation and global LV function obtained by using 3D imaging modality.

Methods: Out of 598 persons who were referred to 64 slices MSCT, the total of 121 patients (age 50 to 69 years, 65% males) with chronic coronary artery disease entered the study. Total Agatston (CAC) score was calculated. Non invasive coronary angiography was performed to detect significant lesions (> 50% diameter reduction). Global LV function was assessed (ejection fraction, stroke volume, cardiac output, LV volume, LV mass, end-systolic and end-diastolic volume). C-reactive protein and leucocytes count were determined.

Results: All patients had coronary artery calcifications. CAC score between 1 and 100 was detected in 74% of patients, between 101 and 399 in 21%, between 400 and 999 in 25%. The total of 25% had CAC score above 1000. Significant lesions were detected in 16% of patients. There was a correlation between CAC and global left ventricular function. Higher CAC correlated with lower ejection fraction (p=0.01), stroke volume (p=0.05), end -systolic volume (p=0.01) and end-diastolic volume (p=0.05). Also, CAC correlated with years of age, male gender and leucocytes count (p=0.05). No correlation was found between CAC and C-reactive protein.

Conclusions: The results of our study suggest that coronary artery calcification is related to global left ventricular function and leucocytes count in patients with chronic coronary artery disease.

Memory Stick

TYPE 2 DIABETES-ASSOCIATED ATHEROTHROMBOSIS: INSIGHTS INTO PATHOGENESIS AND THERAPEUTIC TARGET

Khaled Ahmed[1,2], S. Muniandy[2], I.S. Ismail[3]

[1]Medical Sciences, Faculty of Dentistry, Ibb University, Ibb, Yemen, [2]Molecular Medicine, [3]Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Introduction: Increased atherothrombotic tendency was found in patients with diabetes. N^{ε}-(carboxymethyl)lysine (CML) is a product of proteins glycoxidation and its effect on gene expression of the fibrinolysis regulator; plasminogen activator inhibitor-1 (PAI-1) have not been elucidated.

Objectives: This study was aimed to establish a mechanism by which CML could lead to coronary artery atherosclerosis in patients with type 2 diabetes. In addition, the hypothesis that atherothrombogenic abnormalities are enhanced by CML was elucidated.

Patients and methods: Levels of CML, PAI-1, and tPA in patients with T2DM with and without CAD as well as healthy control subjects were examined. Enhanced oxidative stress and impaired LDL metabolism due to CML were also investigated. Multiplex Real-Time PCR was used to evaluate the gene expression of PAI-1 due to higher levels of CML.

Results: Serum levels of CML and PAI-1 were significantly higher while tissue plasminogen activator (tPA) was lower in T2DM with CAD patients than diabetic patients without CAD or healthy control subjects. Metabolism of LDL from T2DM patients with CAD was completely impaired. Furthermore, CML levels were correlated to the overexpression of PAI-1 genes in T2DM patients compared to healthy subjects.

Conclusions: The present study showed that CML directly enhanced PAI-1 secretion and gene expression in the vascular cells. The hypersecretion of CML might be one of the mechanisms underlying the impaired fibrinolysis and increased tendency for thrombosis observed in patients with T2DM and CAD. Thus, CML may be considered as a therapeutic target in patients with type 2 diabetes-induced atherothrombosis.

Memory Stick

THE ROLE OF FHL2 GENE IN FORMATION OF ATHEROSCLEROTIC LESION

Pao-Hsien Chu[1], H.-I. Yeh[2]

[1]Cardiolgoy, Chang Gung University, Chang Gung Memorial Hospital, [2]Mackay Memorial Hospital, Taipei, Taiwan R.O.C.

Aims: FHL2, a member of the four and a half LIM domain (FHL), may play an important role in the circulatory system and in particular atherosclerosis.

Main methods: To investigate the role of FHL2 in atherogenesis, FHL2-null and wild-type control male mice were fed either a normal chow (NC) or a cholesterol enriched diet (CED).

Key findings: At 3 months post CED, aortic atherosclerotic plaques were observed in both control and FHL2-null mice. Lesions in control mice increased dramatically by 6 months of CED. In contrast, lesion size did not increase during this time in CED fed FHL2-null mice. Relative to control mice on a NCD, control mice on a CED exhibited lower circulating nitric oxide (NO) levels, and decreased expression of connexin37 (Cx37) and Cx40 in aortic endothelium. In contrast, FHL2-null mice on a CED maintained similar levels of circulating NO as FHL2-null mice fed a NCD. Cxs levels in aortic endothelium of FHL2-null mutants on a NCD were lower relative to control mice on a NCD, and did not decrease with CED.

Significance: Our data demonstrate a role for FHL2 in atherogenesis, the regulation of circular NO release, and expression of gap junctions within aortic endothelium.

Memory Stick

PMN MEMBRANE FLUIDITY, CITOSOLIC CA2+ CONTENT AND BETA2-INTEGRIN PATTERN IN VASCULAR ATHEROSCLEROTIC DISEASE

G. Caimi, **Eugenia Hopps**, B. Canino, M. Montana, R. Lo Presti

Dipartimento di Medicina Interna. Malattie Cardiovascolari e Nefrourologiche, Università di Palermo, Palermo, Italy

In atherosclerosis and in diabetes mellitus (DM) polymorphonuclear cells (PMN) play a role in the organ injury. The PMN-mediated tissue damage can be exacerbated by a PMN functional abnormality and among the parameters that reflect this PMN dysfunction we can include membrane fluidity and cytosolic Ca^{2+} content. Both leukocyte-endothelium and leukocyte-platelet interactions, mediated especially by β_2-integrins, are involved in atherogenesis. We evaluated PMN membrane fluidity and cytosolic Ca^{2+} content in subjects with vascular atherosclerotic disease (VAD) with (n=52) and without DM2 (n=92), subdivided according to the extent of vascular disease and the examination of the PMN integrin pattern (CD11a, CD11b, CD11c and CD18) at baseline and after activation in a subgroup of VAD subjects with (n=21) and without DM (n=27). There was no difference in membrane fluidity between controls and VAD subjects, mono or polyvascular, while the cytosolic Ca^{2+} content was increased in VAD subjects (mono and polyvascular). The baseline evaluation of the integrin pattern showed that CD11a and CD18 were increased, while CD11b and CD11c were decreased. After activation we observed, in VAD subjects with and without DM, a decrease of CD11a and an increase of CD11b; we noted also a decrease of CD18 in VAD subjects without DM and an increase of CD11c in those with DM. In conclusion, the increase in PMN cytosolic Ca^{2+} content and the expression of PMN integrins found in VAD patients are markers of PMN activation; the investigation on adhesion molecules has potential therapeutical implications.

Memory Stick

THE ROLE OF NATURAL PROANTHOCYANIDINS ON THE IMPROVEMENT OF INFLAMMATORY MARKERS IN DIABETIC RATS

Magda Badescu[1], R. Furnică[1], L. Bădescu[2], R. Lupuşoru[1], M. Ciocoiu[1]

[1]Pathophysiology, [2]Cell and Molecular Biology, University of Medicine and Pharmacy 'G.T.Popa' of Iasi, Iasi, Romania

Object of study: The understanding of the concept of inflammation in diabetes-accelerated atherosclerosis can be used practically to predict future cardiovascular risk by evaluating inflammatory biomarkers and to design clinical trials making inflammation as a therapeutic target. Plant-derived proanthocyanidins may exert beneficial effects on diabetes mellitus, in part, because of their antioxidant properties.

Method: The benefits of the proanthocyanidins extracted from the black grapes seeds (BGSP), have been shown by using as experimental model the streptozotocin-induced diabetes mellitus on the Wistar white rats. The streptozotocin was administered in a single dose of 60 mg/Kg body mass, intraperitoneal. The vegetal proanthocyanidins (BGSP) were administered under the form of water solution, in a dose of 0,028 g/KG body mass, p.o. (through tube feeding), every two days, for a period of 12 weeks.

Results: Polyphenols administered to STZ rats reduced blood glucose concentration more that 45% after three weeks of treatment. Interleukin 1beta levels were significantly increased in diabetic compared to witness group. After the polyphenols administration, the values decreased. Regarding the levels of ceruloplasmin ($p< 0,001$), fibrinogen ($p< 0,001$) and uric acid ($p< 0,01$), similar findings were noted. Histologically, in pancreatic and hepatic tissue from group DM+P, there were observed degenerative and inflammatory phenomena associated with regenerative phenomena.

Conclusions: The proanthocyanidins extract, with a flavonoidic content of 30,8% in rutoside units out of which 18,3% are antocyans, improves significantly ($p< 0,001$) the inflamatory phenomena and have a protective effect against diabetes microangiopathy.

Keywords: Diabetes mellitus, grape seeds, proanthocyanidins.

Memory Stick

OXIDATIVE STRESS MODULATION IN CARDIOVASCULAR DIABETIC DAMAGES BY POLYPHENOLS SUPPLEMENTATION

Manuela Ciocoiu[1], C. Bădescu[2], C. Boldea[1], M. Bădescu[1]

[1]Pathophysiology, [2]Internal Medicine, University of Medicine and Pharmacy 'G.T.Popa' of Iasi, Iasi, Romania

Introduction: The present study concentrates on demonstrating the biological active characteristics of polyphenols extracted from the *Sambucus nigra* fruits.

Material and method: We used 4 groups of Wistar rats: Group I (W) - witness group; Group II (DM) - experimental induced diabetes with streptozotocin 55 mg/kbw.; Group III (P) - received a natural polyphenols solution, 0.045 mg/kbw, for 16 weeks; Group IV (DM+P) - received polyphenolic extract, in the same dose as group III, for 4 weeks before the induced diabetes and then for 12 weeks after the induced diabetes.

Results: Mean hyperglycemia in rats suffering from diabetes and without polyphenolic protection increased progressively. Due to the polyphenolic protection, the serum activity of glutathione peroxidase is more intense for the DM+P group as compared to the DM group. The serum activity of superoxide dismutase for the diabetic group has significantly low values as compared to the ones registered for W and DM+P groups. The obtained results highlight a significant improvement ($p< 0.001$) in the antioxidative capacity of the serum in diabetic rats treated with natural polyphenols, bringing back to normal the concentration of reduced glutathione, as well as an important decrease in the serum concentration of malondialdehyde, thus proving an important decrease of the lipidic peroxides in serum.

Conclusions: The polyphenol-enriched extract from *Sambucus nigra* do reduce the lipids peroxides, do neutralize the lipid peroxil radicals, and by this may prevent the long term cardiovascular consequences of experimental diabetes.

Memory Stick

NONPHARMAKOLOGICAL TREATEMENT OF OBESITY AT PATIENTS WITH METABOLIC SYNDROME

Pavol Hlubik[1], H. Stritecka[1], J. Hlubik[2]

[1]Military Hygiene, University of Defence, Hradec Kralove, [2]Gerstner Laboratory, Czech Technical University, Prague, Czech Republic

According to the data of WHO Risk of development of non-infectious diseases (obesity, hypertension, diabetes mellitus II type…) of mass occurrence correlates with the age of monitored individuals. Nowadays it is noted that not only the life-expectancy is prolonging, but also the number of individuals in higher age groups is increasing in the whole Europe including the Czech Republic. Especially obesity partakes of risk increase of metabolic diseases: insulin resistance, small dense oxide LDL, higher level of TAG and lower level of HDL…

The aim of study was evaluate effect of dietary supplement to body weight reduction. As test drug was used 1 tablet of Sternax (50 mg dry extract from leuzey, 100 mg caffeine) per day during free month's therapy with dietary restriction and increasing of physical activity, too. Measurement of body composition, body weight, waist and lipid profile, GLU and uric acid was done at beginning and after three months.

Change of anthropometrical parameters was statistical significant bud change o f biochemical parameters (TCHOL, HDL, LDL, TAG, GLU, uric acid) was statistically non-significant.

Using dietary supplements to be one of the ways in body weight reduction, but must be always physical activity and suitable diet supplemented.

Supported by MO FVZ 0000502

Memory Stick

ASSOCIATION BETWEEN NEWLY ONSET ORAL ANTICOAGULANT TREATMENT WITH PHENPROCOUMON AND SERUM LEVELS OF THE VITAMIN-K DEPENDENT CALCIFICATION INHIBITOR MATRIX GLA-PROTEIN

Ralf Koos[1], V. Brandenburg[1], N. Marx[1], L. Schurgers[2]

[1]Department of Cardiology, Medical Faculty, RWTH Aachen University, Aachen, Germany, [2]Cardiovascular Research Institute and VitaK, Maastricht University, Maastricht, The Netherlands

Introduction: Matrix Gla protein (MGP) is a vitamin K-dependent protein. Recently, the uncarboxylated (inactive,uc) form of MGP was associated with vascular tissue calcification. We aimed to investigate the relationship between a newly initiated oral anticoagulant (OAC) treatment with the Vitamin-K antagonist phenprocoumon and serum levels of uncarboxylated MGP compared to a control group with aspirine therapy.

Methods: We performed a prospective study in 43 non-dialyzed patients (mean age 61±10 years) with newly initiated OAC-therapy with phenprocoumon (n=21) or aged-matched subjects with aspirine therapy (n=22). In all patients circulating ucMGP serum levels were measured at baseline as well as after 1 month and 3 months follow-up.

Results: At baseline no significant difference in ucMGP-levels between patients with newly initiated OAC-therapy (1003±526pM) and control subjects with aspirine therapy (842±214pM, p= 0.40) was detected. At 1 month follow-up, patients with OAC-therapy demonstrated significantly increased ucMGP-levels (1904 ± 402pM) compared to the aspirine group (628 ± 223pM, p< 0.001). At 3 months follow-up, a further increase in ucMGP levels in patients with OAC-treatment (2316 ± 344pM) compared to the aspirine group (685 ± 378, p< 0.001) as well as to study inclusion (1003 ± 526pM, p=0.003) was observed.

Conclusion: The present study suggests that newly onset OAC-therapy with phenprocoumon is associated with increased serum levels of inactive,uc-MGP indicating calcification inhibitor deficiency compared to a reference population with aspirine therapy. Long-term follow-up data are needed to evaluate if newly onset OAC-therapy may be linked to an increased progression of coronary and valvular calcification.

Memory Stick

MIDTERM FOLLOW UP OF ARTERIAL CORONARY BYPASS GRAFTING USING THE RADIAL ARTERY VERSUS BILATERAL INTERNAL THORACIC ARTERIES

Jeannette Schönebeck, B. Reiter, O. Haye, D. Böhm, F. Wagner, H. Reichenspurner

KLinik für Herzchirurgie, Universitäres Herzzentrum Hamburg/ Eppendorf, Hamburg, Germany

Objective: Arterial revascularisation using bilateral internal thoracic arteries (BITA) vs unilateral internal thoracic artery (LITA) and radial artery (RA) were compared retrospectively in a case-matched study.

Methods: 568 patients from January 2003 to December 2005 received either BITA (group A, n=116patients) or LITA and RA (group B, n=452patients). Patients were identified from our surgical database. Patients and referring cardiologists were interviewed by written questionnaires.

Results: Significant differences were found in mean age 62.0±8.9 vs.64.4±8.6years ($p< 0.05$), Body Mass Index 26.3±2.9 vs. 28.2±4.5 ($p< 0.05$), Euroscore 3.9±3.8 vs. 4.6±5.5 ($p< 0.05$) and D. mellitus 83.6% vs. 71% ($p< 0.001$) (group A vs. B respectively).

No significant differences were found in rethoracotomy due to bleeding, myocardial infarction and low cardiac output. Mean follow up was 50 months with an overall mortality due to cardiac events of 6.3% group A vs. 7.4% group B. Angiography was performed in 12.5% vs. 16%($p>0.5$) (group A vs. B respectively) due to angina (33%), NYHA class deterioration (33%) and without reliable reasons (33%). Freedom from angina was 87% group A and 84% group B ($p>0.5$). In 60% of angiographies in group A and 40 % in group B no bypass pathology could be found, occlusions of arterial grafts were provable in 25 patients (19RA) in group B vs. 3 patients (3RIMA) in group A ($p< 0.01$).

Conclusions: Both groups showed excellent clinical results in term of freedom of angina and cardiac events. There was a significant trend to a higher bypass occlusion rate in RA group.

Memory Stick

FIBRINOGEN BETA VARIANTS CONFER PROTECTION AGAINST CORONARY ARTERY DISEASE IN A GREEK CASE-CONTROL STUDY

Eirini Theodoraki[1], T. Nikopensius[2], J. Suhorutšenko[2], M. Dimitriou[1], V. Peppes[3], G. Kolovou[4], N. Zakopoulos[3], D. Richter[5], A. Metspalu[2], G. Dedoussis[1]

[1]Harokopio University, Athens, Greece, [2]University of Tartu, Tartu, Estonia, [3]University of Athens Medical School, [4]Onassis Cardiac Surgery, [5]Athens Euroclinic, Athens, Greece

Background and aim: Although plasma fibrinogen levels are related to cardiovascular risk, data regarding the role of fibrinogen SNPs in coronary artery disease (CAD) etiology remain inconsistent. The aim of the present study was to investigate the effect of fibrinogen A (FGA), fibrinogen B (FGB) and fibrinogen G (FGG) gene SNPs and haplotypes on susceptibility to CAD in a Greek population.

Methods: Cases (n=305) were subjects presenting acute coronary syndrome or CAD defined as >50% stenosis in at least one of the three main coronary vessels (81,6% males) (age: 60,3±14,8 years). Controls (n=305) were subjects with normal coronary vessels assessed by coronary angiography or with negative stress test (70,2% males) (age:63,1±11,4 years). 3 SNPs were selected for FGA, 7 SNPs for FGB and 3 SNPs for FGG genes using Carlson's algorithm. Genotyping was performed using APEX-2 method. PLINK 1.2 software was used to perform logistic regression analysis, before and after adjustment for potential confounders. THISEAS software was used to estimate haplotype effects.

Results: From all SNPs and haplotypes tested only rs1800787 and rs1800789 SNPs in FGB gene were associated with CAD, even after adjustment, in the recessive model (OR=0.42, 95%CI: 0.19-0.90, p=0,026 and OR=0.44, 95%CI:0.21-0.94, p=0.039, respectively).

Conclusions: FGA and FGG SNPs, as well as FGA, FGB, FGG and FGA-FGG haplotypes do not seem to be important contributors to CAD in our sample. On the contrary, FGB rs1800787 and rs1800789 SNPs seem to confer protection to disease onset lowering the risk by about 50% in homozygotes for the minor alleles.

Memory Stick

IMMEDIATE EFFECT OF INTENSIVE ATORVASTATIN TREATMENT ON LIPID LEVELS IN PATIENTS WITH ACUTE CORONARY SYNDROME (ACS)

Dagmar Vondrakova, P. Ostadal, A. Kruger

Na Homolce Hospital, Prague, Czech Republic

Introduction: Statin therapy decreases mortality and incidence of coronary events. Recently it has been reported that spontaneous lipid levels remain clinically stable during ACS.

Methods: We have analyzed group of 114 patients with ACS. Atorvastatin 80 mg was administered at admission and then once daily for the rest of hospitalization. The total cholesterol (TC), LDL-cholosterol (LDL), HDL-cholesterol (HDL), and triglycerides (TG) were measured at admission (D0), and then every other morning of hospitalization (D1, D2).

Results: The mean entry values (D0) of TC, LDL, HDL and TG (mmol/L) were 5.24, 3.26, 1.07 and 1.31, respectively. The therapy with atorvastatin 80 mg resulted in a decrease of TC levels in the first morning (D1) by 6.1% and on the second morning (D2) by 13.2% ($p < 0.001$ for all comparisons with the entry value D0); LDL was decreased by 5.8% (D1) and 15.6% (D2) ($p < 0.001$ vs. D0); the HDL was decreased by 7.5% (D1) and 12.1% (D2) ($p < 0.001$ vs. D0). In contrast, the TG level was higher in the first morning (D1) by 20.6% and in the following morning (D2) by 25.5% ($p < 0.05$ vs. D0).

Conclusion: We have shown that intensive statin therapy started at admission in ACS patients has a highly significant, immediate effect on all monitored lipid levels.

Since TC and LDL levels were decreased as predicted, reduction in HDL and increase in TG levels suggest different acute effect of high-dose statin on lipid levels in comparison with long-term treatment of ACS patients.

Memory Stick

PSORIATIC ARTHRITIS AND ATHEROSCLEROSIS: **WHAT IS THE LINK?**

Anna Abou-Raya, S. Abou-Raya

Internal Medicine, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Background: Inflammation plays a role in the pathogenesis of psoriatic arthritis (PsA), a chronic inflammatory disease and also in the pathogenesis of atherosclerosis. Cardiovascular disease (CVD) is an important cause of morbidity and mortality in chronic inflammatory disorders.

Objectives: To evaluate the prevalence of subclinical atherosclerosis in PsA patients and to correlate it with inflammatory markers and disease activity.

Methods: The study population consisted of 56 PsA patients with no previous history or clinically overt CVD and 38 healthy age-sex and traditional cardiovascular risk factors matched controls. Laboratory measurements included: hsCRP, fibrinogen, lipid profile, IL-6 and TNF-alpha. Carotid intima media thickness (CIMT) was assessed by Doppler examination and the presence of plaques documented.

Results: PsA had a higher prevalence of subclinical atherosclerosis. Seventeen of the 56 patients (30%) demonstrated subclinical atherosclerosis. PsA patients had a significantly higher CIMT than in controls [1.14(0.29) mm versus 0.80(0.20) mm. Levels of IL-6 and TNF-alpha were significantly higher in patients than in controls. CIMT correlated with disease duration, disease severity, IL-6 and TNF-alpha respectively.

Conclusion: PsA patients have an increased prevalence of subclinical atherosclerosis. Chronic systemic inflammation appears to be the link between PsA and atherosclerosis. PsA patients are thus at an increased risk of accelerated atherosclerosis and cardiovascular morbidity and mortality. Thus there should be regular screening for subclinical CVD. Furthermore, it is imperative to control inflammation to protect against the development of CVD in PsA patients.

Memory Stick

PERIPHERAL ARTERY DISEASE AND CARDIOVASCULAR MORBIDITY IN RHEUMATOID ARTHRITIS

Suzan Abou-Raya, A. Abou-Raya

Internal Medicine, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Background: Studies of the central arteries in RA patients have shown an increase in the thickness of the intimal layer, suggesting that RA predisposes to atherosclerosis and cardiovascular disease (CVD). The extent of peripheral arterial atherosclerosis however, remains unestablished in these patients.

Objectives: To determine the prevalence and extent of peripheral artery subclinical atherosclerosis in RA patients with no previous history of CVD and the association with clinical and inflammatory parameters.

Methods: Sixty four consecutive RA patients with no previous history of CVD and 37 age-sex-body mass index and traditional risk factors matched controls were recruited. Both patients and volunteers were chosen to be non-smokers. Biochemical measurements included glucose levels, lipid profile, hsCRP and fibrinogen. Peripheral atherosclerosis was assessed using the ankle-brachial blood pressure index (ABPI).

Results: In RA patients, one or more abnormal arteries occurred in 19 of the 64 patients (30%) versus 2 of the 37 control subjects (5%), $p = 0.002$. A significant correlation was observed between ABPI and disease duration, disease severity, hsCRP, and fibrinogen respectively.

Conclusion: The findings demonstrate an increased prevalence of an abnormal ABPI in RA patients which is an indication of subclinical atherosclerosis and consequently increased risk of CVD. The simple, relatively cheap, non-invasive tool, namely ABPI, appears to be useful in assessing peripheral arteries and peripheral artery disease in RA patients and in identifying patients who are at an increased risk of atherosclerosis and further future CVD. It is imperative that peripheral artery disease be not overlooked in RA patients.

Memory Stick

FETUIN- A: AN ADDITIONAL MEDIATOR BETWEEN CHRONIC INFLAMMATION AND CARDIOVASCULAR DISEASE IN RHEUMATOID ARTHRITIS?

Suzan Abou-Raya[1], A. Abou-Raya[1], M. Helmii[2]

[1]Internal Medicine, Faculty of Medicine, University of Alexandria, [2]Biochemistry, Medical Research Institute, Alexandria, Egypt

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disorder, with increased cardiovascular (CVS) morbidity is. Furthermore, atherosclerosis like RA, is characterized by chronic inflammation and increased mineral mobilization. Fetuin-A is a circulating negative acute phase protein of the cystatin superfamily of cysteine protease inhibitors involved in vascular pathology.

Objective: To evaluate the level of fetuin-A in RA patients and to assess its association with subclinical atherosclerosis and the inflammatory state in RA.

Methods: 87 RA patients and 46 healthy volunteers matched for age, sex, body mass index, arterial blood pressure were studied. All subjects were without CVS symptoms or clinically overt CVS disease. Serum Fetuin-A levels were determined by ELISA. High resolution B- mode ultrasound was done to compare carotid artery intima media thickness (CIMT) between RA patients and controls. Plain X -rays of the chest were used to assess the presence of aortic calcifications.

Results: In RA patients, serum fetuin-A levels were significantly lower compared to controls (287± 67.1 versus 449±56.4 µg/ml, $p < 0.001$). Mean CIMT was significantly different in RA patients compared to controls. Vascular calcifications were detected in 14% of patients. There was a significant inverse correlation between fetuin-A levels and CIMT.

Conclusions: The findings of significantly lower levels of fetuin-A and the association of fetuin-A with inflammatory markers, lipid parameters and CIMT in RA patients suggest that reduced fetuin-A levels may be indicative of a chronic inflammatory state and may be associated with increased risk of subclinical atherosclerosis and thus increased CVS risk in RA patients.

Memory Stick

MANAGEMENT OF PATIENTS WITH PRIMARY HYPERCHOLESTEROLAEMIA AND MIXED DYSLIPIDAEMIA BY MEANS OF AN OPTIMIZED BOTANICAL COMBINATION: RESULTS FROM A RANDOMIZED, DOUBLE-BLIND, PARALLEL-GROUP, PLACEBO-CONTROLLED TRIAL

H. Gupta[1], D. Pawar[2], A. Riva[3], E. Bombardelli[3], **Paolo Morazzoni**[3]

[1]Department of Medicine, Grant Medical College Mumbai, [2]Department of Pharmacology, Drug Research Laboratory, Mumbai, India, [3]Scientific Department, INDENA S.p.A., Milan, Italy

Background and objectives: The objective of this study was to compare the efficacy and tolerability of an optimized botanical combination (CHOLACTIV®) against Placebo in the management of patients with primary hypercholesterolaemia and mixed dyslipidaemia. CHOLACTIV® contains in a rational proportion four different botanical derivatives (policosanol, tomato extract, orally bioavailable grape procyanidins, *Oenothera biennis* oil) endowed with complementary biological properties targeted to cholesterol control and vasoprotection.

Subjects and methods: This double blind, randomized, parallel group, placebo-controlled study in dyslipidemic patients (n=60) consisted of 6 weeks treatment period following 4-week baseline period and 2 week post-treatment follow-up period.

Results: At baseline both the groups were comparable to each other. A significant reduction in the LDL (17.33% from baseline) and TC (13.38% from baseline) value over the treatment period was observed with CHOLACTIV®. The treatment also resulted in the reduction in CRP, MDA and SOD values which are oxidative stress indices.

Conclusion: The rational combination of policosanol, tomato extract, orally bioavailable grape procyanidins and *Oenothera biennis* oil is effective and safe in lowering the elevated LDL and TC values. It is also effective in lowering the oxidation indices values; however, an additional long term study in a larger population has to be conducted in order to confirm these findings.

Memory Stick

ATHEROSCLEROTIC RISK BURDEN ASSESSMENT ACCORDING AS BIFURCATION ANGLE USING COMPUTED TOMOGRAM, IVUS AND FLUID DYNAMICS

Byoung Kwon Lee[1], H.M. Kwon[1], T.H. Kim[2], H.W. Roh[3], C.Y. Kim[4]

[1]Internal Medcine, Cardiology, [2]Diagnostic Radiology, Gangnam Severance Hospital, Yonsei University, [3]IVAI GD3 Co., [4]Cybermed Co., Seoul, Republic of Korea

Background: Atherosclerotic coronary stenosis might be affected by hemodynamic factor, such as oscillatory shear stress. The purpose of this study is investigating the flow characteristics and distributions of the hemodynamic wall parameters (HWP) and comparing atheroma volume (AV) according as bifurcation angle (BA).

Methods: Coronary bifurcation models between left anterior descending artery and first diagonal branch according as different angle were made from computed tomogram (CT) data. HWP, such as time averaged wall shear stress (TAWSS), oscillating shear index (OSI), and wall shear stress (WSS) distributions in coronary bifurcation model were calculated under different BA (30, 45, 60, and 90^o). AV was analyzed quantitatively with CT and intravascular ultrasound (IVUS) from patients with stable angina (n=89). AV was compared according as BA.

Results: The bigger the BA, the lower TAWSS and the higher OSI at SB [TAWSS(Pa): 0.133, 0.020, 0.045, 0.060, OSI: 0.309, 0.472, 0.442, 0.469 at 30, 45, 60, and 90^o, respectively]. HWP in 30^o were significantly different comparing with that of other larger angle. Low shear zone was widening when the BA increasing. The mean AV of side branch in small BA (n= 40, < 45^o) was significantly smaller than large BA (n=49, >45^o) (12.5±6.2 mm^3 vs. 25.6±10.7 mm^3), p = 0.035) and correlated positively (r^2=0.15, p< 0.05).

Conclusion: Over 45^o of BA might have more hemodynamically atherogenic risk and might have restenosis risk of SB after main branch intervention. We suggest that small BA side branch might have lower risk for atherosclerosis and restenosis.

Memory Stick

AF & PACEMAKERS

Mahmoud Abou Zeid

Mouwassat Hospital, Dammam, Saudi Arabia

232 patients on pacemakers were evaluated for one year to assess the prevalence and the incidence of new cases of AF.

All patients subjected to:

1. Thorough history taking.
2. Full clinical examination.
3. Standard resting 12 leads ECG.
4. Echocardiographic study, with special concern on detecting left atrial diameter, left ventricular function and valvular state.

The results evaluated, calculated, tabulated and statistically analyzed.

Patients were divided according to their atrial rhythm at the time of follow up into two groups:

Group I: Included patients with atrial sinus rhythm at the time of follow up.

Group II: Included patients with AF at the time of follow up.

The study concluded that , Proper mode selection of pacing before implantation of permanent pacemaker considering atrial or atrioventricular sequential pacing {AAI(R) or DDD(R)} rather than VDD pacing should be in a special consideration. Physicians should give a special attendance to atrial rhythm while analyzing an ECG recording of a patient on pacemaker not only to consider pacing and sensing functions of the pacemaker but also to elicit if the atrial rhythm of the patient is sinus or atrial fibrillation. Patients on VVI(R) should receive proper prophylaxis against thromboembolism as they are in high risk of developing AF specially if:

1. Detected atrial fibrillation.
2. They had increased LA diameter.
3. The longer the duration of pacing irrespective to the indications.

Memory Stick

THE SURVEY OF PATIENT´S QUALITY OF LIFE WITH ISCHEMIC HEART DISEASE

Mohsen Taghadosi

Mediical University, Kashan, Iran

Background: This study was conducted on determination of patient quality of life for those Ischemic referring ones to Kashan therapeutic centers in 2007-2008.

Materials and methods: This cross-sectional study was carried out on those referring patients to Kashan therapeuticinfarction per clinical and Para clinical finding or had a history of PTCA or centers. SF-36 Index was used to evaluate their general quality of life and SAQ index was performed to assess the specific quality of life. Per SAQ scoring, the specific quality of life lower than 30 considered as low, 31-50 medium, 51-70 good and grater than 71 was very good. Per SF-36 those scores lower tan 38 had low general life quality, 38-76 medium, 77-115 good andexecellent. The data were analyzed by using K2 and Fisher test.

Findings: Average was 60.5±12.5 years old. From 500 patients, %55.8 were male. Most of the patients had heart of the patients had heart MI history %93.6 were couple, %42.4 with reading and education. %34 had angina pain during the last month. %95.2 had at least one risk factor as follows diabetes, hypertension, and high blood fat. %26.1 had PTCA operation, and %8.7 were under CABG. The average of life quality according to SF-36, 112.5±24 and according to SAQ was 59.8±14.9. The effective features on life quality involve history disease, heart disease, and high age, lack of enough education, educating, MI numbers, and sex.

Conclusion: Most of the patients had medium and good life quality Patients with CABG had better life quality than PTCA ones.

Memory Stick

ROLE OF OBESITY, DIABETES, HYPERTENSION, SMOKING, FATS, CARBOHYDRATES, PROTEINS, SALT, AND WATER ON DEVELOPMENT OF CARDIO VASCULAR DISEASES

Ram Mathur

California Department of Health, Richmond, CA, USA

This hypothesis will be an eye opener to all the researchers in this field. This is the only hypothesis which explains the involvement of obesity, diabetes, hypertension, smoking, fats, carbohydrates, amino acids, salt, and water.

First of all it is the hyperosmolar food that causes CVD by threatening to raise blood temperature. Osaka et al (J Physiol 2001; 523:261-9) have demonstrated that the solutions of glucose, fructose, NaCl, and amino acids individually induce thermogenesis in urethane-anesthetized rats. Hyperglycemia in diabetics produces same physiological affect as infusion of glucose. In short, all the nutrients listed above, when taken in high concentartions at one time will cause thermogenesis in the body. Since, fats do not cause hyperosmolarity , it is not the cause of CVD.

People from Okinawa Japan have the lowest incidence of CVD because their food is watery. They eat Meso soup three times a day i.e. breakfast, lunch and dinner.

Meso soup is composed of mainly water, vegetables, and tiny amount of meat. The Oknawians´ diet is low glycemic as well which does not cause a sudden flux of sugars in blood stream. Similarly, Mediterranean diet is also to some extent is protecting from CVD.

The heat generated from thermogenesis can not dissipate from the body of obese people therefore, they are more prone to CVD. Lean people are usually less suseptable to CVD. Therefore, obesity is not the cause of CVD, it only exacerbates the condition. Similarly, obese smokers are more prone to CVD.

Memory Stick

LONG-TERM PLEIOTROPIC EFFECT OF STATINS UPON NITRIC OXIDE AND C-REACTIVE PROTEIN LEVELS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

Joaquin de Haro[1], S. Bleda[2], A. Florez[2], C. Varela[2], L. Esparza[2], F. Acin[2]

[1]Angiology and Vascular Surgery, Hospital Universitario Getafe, Boadilla del Monte, [2]Angiology and Vascular Surgery, Hospital Universitario Getafe, Getafe, Spain

Objectives: Peripheral arterial disease(PAD) can be regarded as a systemic inflammatory disorder affecting the entire vascular system. In the early clinical stages it is characterized by the deterioration of endothelial function. This study analyzes the plasma nitrite and CRP levels in claudicating patients during 12 months of treatment with statins.

Methods: A prospective study was made of 60 patients with Fontaine grade II, 30 treated with statins for 12 months from the time of diagnosis and 30 without statins as controls. Measurements were made of plasma high-sensitivity CRP(hsCRP) and nitrites at baseline and after one and twelve months.

Results: A significant reduction in nitrite levels was observed after one month of treatment(11.8±7.8 µM vs 5.7±1.8 µM;p=0.0001). This effect didn't persist(9.4±8.9 µM;p=0.27) after one year. hsCRP underwent significant reduction after both one month(13.58±24.00 vs 3.93±3.19;p=0.02) and one year of treatment(4.59±4.94;p=0.05).

Conclusions: Statin therapy reduces the plasma nitrite and CRP levels in patients with peripheral arterial disease from the first month of treatment. The initial effect upon NO bioavailability is not maintained over time, in contrast to what is seen in the case of the inflammatory process.

Memory Stick

SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH PSORIATIC ARTHRITIS: THE IMPACT OF BLOOD PRESSURE

Massimo Puato, M. Zanardo, G. Balbi, M. Zanon, E. Faggin, M. Rattazzi, R. Ramonda, A. Lo Nigro, A. Doria, P. Pauletto

Dipartimento di Medicina Clinica e Sperimentale, Università di Padova, Padova, Italy

Objective: Increased cardiovascular mortality have been observed in rheumatic diseases. We evaluated vascular remodelling in psoriatic arthritis (PsA) according with hypertensive status by studying non-invasively structural and functional properties of arteries.

Method: We studied 41 consecutive patients with PsA (of whom, 48% hypertensives). 40 normotensives healthy subjects (N-C) and 18 hypertensives (HT-C) served as controls. We measured by B-mode ultrasound the carotid intima media thickness (IMT) expressed as mean-IMT (cumulative mean of mean IMT measured in each carotid segment, common, bulb, and internal, bilaterally) and as M-MAX (cumulative mean of maximum IMT). Endothelial function was evaluated by post-occlusion flow-mediated dilation (FMD) of the brachial artery using high-sensitivity ultrasonography. NO-independent vasodilation was evaluated by the response to sublingual glyceril-trinitrate (GTN).

Results: PsA had a higher mean-IMT compared to N-C (normotensives PsA 0.69 mm, hypertensives PsA 0.71 vs N-C 0.60 mm, p=0.005). Hypertensive PsA displayed higher M-MAX (0.91 mm) versus both controls (N-C 0.71 and HT-C 0.77 mm, p=0.007) and normotensive PsA (0.81 mm, p=0.026). FMD was lower in PsA than in N-C (normotensives PsA 5.7%, hypertensives PsA 6.1% vs NC 8.9%, p< 0.0005), whereas there was no difference between hypertensive PsA, normotensive PsA, and HT-C. GTN was similar in all groups.

Conclusions: Subclinical atherosclerosis is enhanced in PsA compared to N-C. In PsA, the hypertensive status exert an additional effect on M-MAX, a parameter of advanced pro-atherogenic remodelling. FMD was reduced in PsA irrespective of hypertensives status. Thus, PsA per se implies a pro-atherogenic remodelling which is enhanced by hypertensive status.

Memory Stick

A NOVEL APPROACH THROUGH ELECTROMAGNETIC FORCES TO ATHEROSCLEROSIS

Reza Paeizi, E. Barzanouni, M. Bokaiyan

Mashhad University of Medical Sciences, Mashhad, Iran

Atherosclerosis is a disease in which deposits of fatty substances such as Low Density Lipoprotein (LDL) particles are formed within the intima of large and medium sized elastic and muscular arteries. The formed plaque causes hardening of the artery walls, reduces arteries flow and it can also completely block blood flow. Although there are several hypotheses that explain the mechanism of atherosclerotic plaque formation and analyze the process mechanical or biochemical approaches, it`s origin has not been completely understood. Our hypothesis approaches this process through a new point of view. It emphasizes on LDL deposition into the intima layer of a coronary artery which is caused by electromagnetic forces produced by flowing electrically charged blood constituents in neighbouring arteries and produced electrical current of heart. The forces act on flowing LDL particles and deflect them, so increase the susceptibility of LDL deposition into the intima layer. Future investigations may help computerizing simulations of electromagnetic forces in the vasculature to reveal highly atherosclerosis-prone regions. Taken together, the role of electromagnetic forces in coronary arteries atherosclerosis can help us design more efficient prophylactic and therapeutic strategies.

Memory Stick

EPICARDIAL ADIPOSE TISSUE FATTY ACID BINDING PROTEIN4 EXPRESSION IS CORRELATED WITH EXTENSION OF CORONARY ATHEROSCLEROSIS IN PATIENTS WITH METABOLIC SYNDROME

Selçuk Görmez[1], A. Demirkan[2], F. Atalar[3], B. Çaynak[4], D. Günay[5], B. Akpınar[4], U. Özbek[3], V. Aytekin[6], N. Yazıcıoğlu[7], A.S. Büyükdevrim[8]

[1]Cardiology Department, Florence Nightingale Hospital, Istanbul, Turkey, [2]Department of Genetic Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands, [3]Department of Genetics, Istanbul University, Institute for Experimental Medical Research, [4]Department of Cardiovascular Surgery, Istanbul Bilim University, Faculty of Medicine, [5]Department of Biochemistry, Florence Nightingale Hospital, [6]Department of Cardiology, Istanbul Bilim University, Faculty of Medicine, [7]Department of Cardiology, Florence Nightingale Hospital, [8]Turkish Diabetes Consortium, Istanbul, Turkey

Objective: Fatty acid binding proteins are a group of cytosolic lipid carriers that coordinate inflammatory and metabolic responses in cells. Adipocyte fatty-acid-binding protein 4 (FABP4) is a member of adipokines family and expressed in adipocytes and macrophages. It has been shown a strong pathophysiological association between FABP4 and metabolic syndrome (MS), diabetes and atherosclerosis. In this study, we investigated the association between FABP4 and the extent of coronary atherosclerosis in patients with MS.

Methods: Between November 2007 and January 2009, 37 patients with MS who were to undergo coronary bypass surgery due to coronary artery disease in our institution were enrolled prospectively to our study. Phenotypic parametres of the study group are presented in Table 1. MS patiens fit the criteria depicted by ATPIII. Patients with pregnancy, chronic renal and hepatic insufficiency, cancer, endocrine and severe psychiatric disease were excluded from the study. Coronary angiography was performed on all of the study patients. The extent of coronary atherosclerosis was assessed by using Sullivan's scoring system. Relative gene expressions (arbitrary unit) of FABP4 in study group was evaluated in epicardial, pericardial and subcutaneous adipose tissue by using quantitative RT-PCR method.

Results: The expression of FABP4 in epicardial adipose tissue was positively correlated with the Sullivan's score (r: 0.53, p=0.002). Contrarily, there were no correlation between pericardial and subcutaneous adipose tissue FABP4 expression and Sullivan's score (r: -0.15, p=0.40, r: -0.03, p=0.88, respectively).

Conclusion: FABP4 expression in epicardial adipose tissue may be responsible for the coronary atherosclerosis in patients with MS.

Memory Stick

PARAMETERS AFFECTING PROGNOSIS OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION. AN AMI REGISTRY

Alexandros Gotsis, I.-A. Kerasidou, A. Gagalis, A. Risggits, D. Theodoridis, A. Koutsogianni, L. Borghi, P. Bozia, M. Konstantarakis, A. Panagiotidou

Cardiology, General Hospital of Komotini, Komotiní, Greece

Objectives: The aim of this registry was to study the epidemiological and clinical characteristics of AMI (according AHA-WHF-ESC statement), in order to both prevent AMI and treat patients effectively. The aim of this study is to identify the parameters that affect positively prognosis of patients hospitalized with AMI.

Methods: We studied 724 patients (506 men, aged 66.6 and 216 women, aged 73.3 years old) hospitalized from January 2005 up to December 2007 with discharge diagnosis of AMI. A detailed medical history was taken emphasizing to risk factors. Statistical analysis was performed by using SPSS 10.0. The methods used were x^2, Mann-Whitney test and Binary Logistic Regression.

Results: Older patients ($p< 0.001$), women ($p=0.008$), patients with previous stroke ($p=0.024$) and patients who did not receive GP IIb-IIIa inhibitors ($p< 0.001$) had increased mortality. Hyperlipidaemic patients ($p=0.059$) and patients with STEMI ($p=0.065$) have a trend to appear increased mortality, but these risk factors do not affect statistically significant patients´ survival after AMI. Based to the results mentioned above, there was an effort to create an algorithm of mortality prediction for patients with AMI by using Binary Logistic Regression. As a result, male sex ($p=0.013$, OP=2.215) and luck of previous stroke ($p=0.019$, OP=2.473) appear to be independent factors of positive prognosis.

Conclusions: Older patients, women, patients with previous stroke and patients who did not receive GP IIb-IIIa inhibitors during hospitalization had worse prognosis. According to our algorithm of mortality prediction after AMI, men and patients without previous stroke medical history have positive prognosis.

Memory Stick

HIERARCHICAL ANALYSIS OF BIOMARKERS' LEVELS, IN RELATION TO HYPERTENSION STATUS AMONG APPARENTLY HEALTHY PEOPLE

Vassiliki Bountziouka, M. Bonou, A. Evangelopoulos, N. Vallianou, D. Politou, E. Bathrellou, A. Giotopoulou, D. Panagiotakos

Harokopio University, Athens, Greece

Object: To evaluate the ability of various biomarkers in relation to hypertension among apparently healthy adults.

Methods: 115 men (50±15 years) and 185 women (48±15 years) were randomly enrolled to the study. Discriminant analysis was used to assess the biomarkers that classify normotensive from treated or untreated hypertensive.

Results: 74% (45±14 yrs) of the participants were normotensive, 14% (55±14 yrs) were untreated hypertensive and 12% (64±10 yrs) were hypertensive (i.e. SBP/DBP >140/90 mmHg or use of treatment). Untreated hypertension prevailed in 14% in both genders, while 15% of males and 10% of females were treated hypertensive. Education status was 14±4.7 yrs in normotensive, 10±5.5 yrs in treated and 11±5.7 yrs in untreated hypertensives ($p< 0.001$). Hierarchical analysis revealed that among the investigated biomarkers, gGt (lambda =0.949, p=0.001), LDH (lambda =0.951, p=0.001), uric acid (lambda =0.961, p=0.004), cysteine (lambda=0.963, p=0.005), ALP (lambda =0.974, p=0.026), urea (lambda =0.974, p=0.027) and C-RP (lambda =0.982, p=0.078) were the factors that best classified participants in the aforementioned sub-groups. Fisher's Discriminant equations revealed that cysteine, creatinine and C-RP were the factors that best characterized untreated hypertensives. The explained variability of the developed model was 30% ($p< 0.001$), while 63% was the correct classification rate.

Conclusions: 50% of hypertensive individuals were untreated, with increased levels of biomarkers related to renal function. The latter, in addition to the high prevalence of hypertensives among low educated people, makes this group at extremely high-risk for developing atherosclerotic and other diseases. Health care practitioners should focus their interest in this group.

Memory Stick

CORRELATION OF IIB-IIIA INHIBITORS´ ADMINISTRATION AND PROGNOSIS OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION. AN AMI REGISTRY

Alexandros Gotsis, I.-A. Kerasidou, A. Gagalis, A. Risggits, D. Theodoridis, A. Koutsogianni, L. Borghi, P. Bozia, M. Benechoutsou, A. Panagiotidou

Cardiology, General Hospital of Komotini, Komotiní, Greece

Objectives: The aim of this registry was to study the epidemiological and clinical characteristics of AMI (according AHA-WHF-ESC statement), in order to both prevent AMI and treat patients effectively. The aim of this study was to correlate GP IIb-IIIa inhibitors' administration and prognosis of these patients.

Methods: We studied 724 patients (506 men, aged 66.6 and 216 women, aged 73.3 years old) hospitalized in our department from January 2005 up to December 2007 with discharge diagnosis of AMI. A detailed medical history was taken emphasizing to CAD risk factors and previous cardiovascular events. Statistical analysis was performed by using SPSS 10.0. The methods used were x^2 and Mann-Whitney test.

Results: GP IIb-IIIa inhibitors were administrated in 41% of patients (29.5% of patients with STEMI and 52% of patients with NSTEMI). GP IIb-IIIa inhibitors' administration was more frequent among younger patients, men with NSTEMI (p=0.023), patients without previous stroke and non-hypertensive patients. There is not statistically significant difference in GP IIb-IIIa administration in diabetic and non-diabetic patients and those with or without previous AMI. Furthermore, statistical analysis proved no significant difference in frequency of minor hemorrhage events, after GP IIb-IIIa inhibitors' administration. A detailed presentation of all above mentioned data appears in the following table.

	AMI (total) n=724 (p value)	NSTEMI n=375 (p value)
Younger patients	<0.001	<0.001
Without stroke history	0.004	<0.001
Without hypertension history	0.044	0.002
Without diabetes history	0.152	0.052
Without previous MI	0.816	0.026
Mortality	<0.001	0.012
Minor hemorrhage events	0.071	0.037

[AMI and GP IIb-IIIa inhibitors' administration]

Conclusions: GP IIb-IIIa inhibitors were administrated in both high and medium risk patients without statistically significant difference in frequency of use. Patients who received GP IIb-IIIa inhibitors after AMI had better prognosis while no difference in minor hemorrhage events occurrence was observed.

Memory Stick

EFFECTS OF FLUVASTATIN ON TUMOR DEVELOPMENT AND PARAOXONASE-1 ACTIVITY IN RATS FED WITH CHOLESTEROL-RICH DIET

János Padra[1], P. Kertai[2], G. Fóris[1], P. Fülöp[1], I. Seres[1], A. Oláh[3], Z. Balogh[1], G. Paragh[1]

[1]First Department of Medicine, Medical and Health Center, University of Debrecen, [2]Institute of Preventive Medicine, Medical and Health Science Center, University of Debrecen, [3]Department of Clinical Biochemistry and Molecular Pathology, Medical and Health Center, University of Debrecen, Debrecen, Hungary

Recently human follow-up studies and animal experiments suggested a relationship between hypercholesterolemia (HC) and cancer. Moreover, statins may be beneficial for the prevention and treatment of cancer.

Objectives: We aimed to establish an animal model, appropriate for the simultaneous study of HC and tumor development.

Methods: The experiments were carried out on male and female FLF1 rats after 6 weeks of cholesterol-rich diet (CRD). Fluvastatin treatment and tumor inoculation were applied 21 days after the beginning of CRD. Tumor cells (He/De hepatocellular cell line) were inoculated under the renal capsule. At the 42th day blood samples were taken and tumor weights, serum lipids and PON1 activities were determined.

Results:

1.CRD caused an elevation of LDL-C and HDL-C levels.

2. The PON1 activity was higher in male than in female rats, and the CRD decreased PON1 activity both in male and female rats, which was not altered by gonadectomy.

3. In female F1 hybrids the increase in serum LDL-C and HDL-C levels were more pronounced than in male rats. Gonadectomy had no effect on these sex-dependent differences.

4. CRD caused a significant increase in the development of the primary He/De tumor and its metastases. Tumor development had no effect on the elevated serum LDL-C and HDL-C levels, however, PON1 activity was significantly decreased.

5. Fluvastatin administration decreased both serum LDL-C and HDL-C levels and tumor development in the same rat.

Conclusion: Our model is appropriate to study the effect of therapeutics against both hypercholesterolemia and cancer in the same rat.

Memory Stick

PLASMA FATTY ACID COMPOSITION IN PANCREATIC CARCINOMA

Marek Vecka, J. Macášek, T. Krechler, M. Urbánek, M. Zeman, A. Žák

IVth Dept Int Med, 1st Medical Faculty, Charles University, Prague, Czech Republic

Background: Pancreatic carcinoma (PC) is one of leading causes of gastrointestinal malignities in the Czech Republic. The nutrition factors playing a role in PC onset and development also include higher input of fat with lower intake of polyunsaturated fatty acids (PUFA) of n-3 family.

Materials and methods: The study included 62 patients (33M/29F) with pancreatic carcinoma and 39 control individuals (14M/25F). The fatty acid (FA) profile in plasma lipid classes was analysed by gas chromatography.

Results: In plasma phospholipids, we found higher content of saturated and monounsaturated FA in the PC group, caused by higher contents of palmitic and oleic acids; changes in PUFA content included lower ratio of linoleic acid, which was reflected in decreased proportion of PUFA n-6 sum (all $p < 0.0001$) in PC group. The contents of eicosapentaenoic as well as α-linolenic acids were in PC group lower (both $p < 0.0002$). Lower content of linoleic acid was observed also in cholesteryl esters, whereas raised content of monounsaturated FA was found in both triacylglycerols and cholesteryl esters.

Correlations of FA within the PC group revealed positive relationships between eicosapentaenoic acid and activity of cholinesterase, concentrations of total protein, albumin as well as with prealbumin (all $p < 0.05$).

Conclusions: The changes in FA profile in patients with pancreatic cancer are probably of multifactorial origin. The factors involved are pathogenesis of tumor cachexia, enhanced turnover of free FA, inhibition of lipoprotein lipase and activation of *de novo* lipogenesis.

Study was supported by the Research Project MSM 0021620820

Memory Stick

LARGE-SCALE PHENOTYPING OF *EMP3*-KNOCKOUT MICE AT THE GERMAN MOUSE CLINIC REVEALS A SPECIFIC ROLE OF THE GENE IN IMMUNITY

Zouhair Aherrahrou[1], T. Adler[2,3], V. Gailus-Durner[2], H. Fuchs[2], D.H. Busch[3], A. Wagner[1], M. Hrabé de Angelis[2,4], H. Schunkert[1], J. Erdmann[1]

[1]Department of Medicine II, Universität zu Lübeck, Lübeck, [2]German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, [3]Institute for Medical Microbiology, Technische Universität München, München, [4]Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising, Germany

Introduction: Epithelial membrane protein 3 (*EMP3*) was previously reported to be involved in immunological reactions and in tumor as potential suppressor, as well as a candidate gene for cardiac dystrophic calcification (DCC). Given its assumed role in different disorders, we aimed to generate and perform a generalized phenotypic screening of *EMP3*-knockout mice.

Methods: Knock-out targeting strategy was used to generate knockout mice for *EMP3*. Large-scale phenotyping screens were performed at the German Mouse Clinic to obtain a standardized and comprehensive way of phenotyping. The phenotype screens involved tests for nearly 320 parameters in different screening areas. For DCC, screening was performed in our laboratory using the freeze-thaw injury method.

Results: *EMP3*-KO mice are viable and fertile. Under baseline conditions, an aberrant immunological phenotype was found with nearly 70% penetrance in mutant male mice. Specifically, a lower frequency of T cells and an inverse trend in B cells was observed. Also a higher frequency of B cells was seen in female mutant mice. For all the remaining screening, no genotype-specific differences were found. Also no calcification deposits were found in the *EMP3*-KO mice as response to injury.

Conclusion: Large-scale phenotypic screening suggests a role of *EMP3* under basal conditions in immunity. Differences in the proportion of various leukocyte subsets from the corresponding wild type were found. Further investigation is ongoing to demonstrate the role of *EMP3* as tumor suppressor in a sensitized model. Finally, we excluded the *EMP3* gene as candidate gene for DCC on the C57BL/6 genetic background.

Memory Stick

METABOLISM OF CHROMIUM COMPOUNDS IN RATS AND HUMANS

Peter Nielsen, K. Blaue, N. Laschinsky, G. Coxilha, N. Friedrich, K. Kottwitz

Department of Biochemistry and Molecular Biology II: Molecular Cell Biology, University Medical Center Hamburg, Hamburg, Germany

Objectives: Chromium supplementation is discussed in the literature as a new way to prevent or treat insulin resistance and diabetes type 2. However, little is so far know on the biochemical mechanisms of Cr^{3+} action, and on the metabolismus of chromium from various pharmaceutical Cr compounds.

Methods: Cr compounds used in food supplementation were labelled with ^{51}Cr and the absorption, retention and organ distribution was studied in rats after oral or parenteral administration. The absorption of Cr-picolinate and Cr-D-phenylalaninate was tested also in human volunteers using ^{51}Cr-whole-body-counting.

Results: Independent from the dose, the 7-d-whole body retention of ^{51}Cr after oral application was rather low (< 1 %) from all compounds tested. Cr-picolinate, the market leader for Cr supplementation, is absorbed significantly better in rats and in also in humans, however, most of its Cr is directly lost into the urine indicating a different absorption mechanism from this stable Cr-complex. Cell culture experiments indicate a passive uptake of Cr in macrophages and hepatocytes. The ^{51}Cr whole-body-retention data in rats can be described in a 3-compartment model with typical half-lives of 0.2, 5, 100 days. The longer half-lives were similar for all Cr-compounds indicating the storage of comparable amounts of Cr in liver, muscle, fat, and bone.

Conclusions: This study shows the limited bioavailability of oral chromium in rats and humans, especially of Cr-picolinate. This stable complex is absorbed as intact molecule to a higher degree compared to more ionic compounds, however, the retention is lower due to higher urine excretion.

Memory Stick

DOES RATIO OF WIDTH TO LENGTH OF LEFT ATRIAL APPENDAGE DETERMINE THE OCCURRENCE OF CEREBROVASCULAR STROKE EVENT?

Maddury Jyotsna[1], S. Kaul[2], R. Narendra Kumar[1], G. Indrani[1]

[1]Cardiology, [2]Neurology, Nizam's Institute of Medical Sciences, Hyderabad, India

Objective: To see the Left atrial appendage size (width and length) in ischemic cerebrovascular accident (CVA) patients.

Design and method: Transesophageal echocardiography (TEE) was done in ischemic CVA patients after initial stabilization. We compared width length ratio(WLR) calculated from the average of width and length of LAA observed in earlier study by John P et al as controls. We chosen this autopsy study as controls , as it is not possible to have TEE data in normal subjects.

Results: Study group includes 143 patients (F:M::37:106) and control group was 400 subjects (F:M::200:200) from the previous study . The mean±std of width and length of LAA in females and males in study group were 1.99±0.67, 2.397±0.58 and 2.19±0.73, 2.49±0.66 respectively. In females, WLR is more (0.83 ± 0.37) in study group than in control group (0.66±0.26) which is statistically significant ($z=4.06$, $P < 0.000001$). So also for males (study group: control group :: 0.88 ±0.39 : 0.71±0.28) which is also statistically significant ($z=7.03$, $P < 0.000001$). So, broad and short LAA could determine the formation and dislodgement of thrombus resulting in embolic stroke.

Conclusion: Higher the WLR of LAA, greater chances of embolic stoke.

Reference: 1. John P. Veinot, Phillip J. Harrity, Federico Gentile, Bijoy K. Khandheria et al Anatomy of the Normal Left Atrial Appendage. A Quantitative Study of Age-Related Changes in 500 Autopsy Hearts: Implications for Echocardiographic Examination *Circulation.* 1997;96:3112-3115.

Memory Stick

EFFECT OF LOVASTATIN THERAPY AND WITHDRAWAL ON SERUM URIC ACID LEVEL IN TYPE II DIABETIC NEPHROPATHY

Nariman Nezami[1], J. Safa[2], H. Argani[1], A. Ghorbanihaghjo[3], A.M. Vatankhah[1]

[1]Drug Applied Research Center, [2]Department of Nephrology, [3]Biotechnology Research Center, Tabriz University (Medical Sciences), Tabriz, Iran

Background: Recent studies have demonstrated that high uric acid level is a major risk factor of nephropathy and cardiovascular events in the patients with type II diabetes. We aimed to evaluate the serum uric acid (UA) level changes following treatment and withdrawal of lovastatin in patients with type II diabetic nephropathy (DN II).

Methods: Thirty male patients with DN II were enrolled in the study. lovastatin, 20 mg/d, was administered for 90 days. Afterwards, lovastatin was withdrawn for the next 30 days. Blood samples were obtained at baseline, after 90 days of the intervention, and 30 days after withdrawal of lovastatin. Serum level of UA was determined by uricase/PAP method (an enzymatic color test). Lipid profile was assessed using commercial reagents.

Results: After 90 days lovastatin intervention, Cholesterol (TC) and low-density lipoprotein (LDL-C) levels significantly decreased and high-density lipoprotein (HDL-C) level increased significantly ($p< 0.001$, 0.010 & 0.008), despite the unchanged level of triglyceride (TG). After 30 days of withdrawal, TC, TG and LDL-C levels were significantly increased ($p< 0.001$, 0.010, < 0.001), but HDL-C level was not changed. Serum levels of UA were not changed during intervention and withdrawal of lovastatin.

Conclusions: Lovastatin have not any effect on serum UA level in patients with DN II, and there is not any correlation between anti-lipidemic effect of lovastatin and its effect on serum UA.

Memory Stick

ROSUVASTATIN MODULATES GLUCOSE AND FFA-INDUCED PRO-FIBROTIC PATTERNS IN HUMAN MESANGIAL CELLS

Anna Solini, E. Santini, C. Rossi, A. Salvati, E. Ferrannini

Department of Internal Medicine, University of Pisa, Pisa, Italy

Introduction: Persistent inflammation contributes to the progression of mesangial expansion toward fibrosis.Statins show anti-inflammatory effects in patients with chronic kidney disease.

Methods: We explored the effect of rosuvastatin (RSV) on inflammatory and pro-fibrotic responses due to exposure to normal (NG) or high glucose (HG) or free fatty acid (FFA) of human mesangial cells (HMC) treated with Angiotensin-II (Ang-II).We measured MMP-2,MMP-9,TIMP-1,TIMP-2 expression (by realtime-PCR,reported as targeted/reference ratio) and activity (by zymography,expressed as AU),and TGFβ,fibronectin and collagen-IV release by ELISA.

Results: At NG,Ang-II dose-dependently downregulated MMP-2 (0.67±0.19 and 0.40±0.10 at 0.5 and 1mM,$p< 0.01$ *vs* basal);RSV partially reversed this effect (1.67±0.12 and 2.5±0.21 with RSV 1 and 5mM,both $p< 0.005$ *vs* Ang-II 1mM).Conversely,TIMP-2 (main MMP-2 inhibitor) and MMP-9 were upregulated by Ang-II (TIMP-2:1.17±0.14 and 1.87±0.18,$p< 0.008$ *vs* basal;MMP-9:2.53±0.50 and 3.33±0.74,$p< 0.05$ *vs* basal) and down-regulated by RSV (TIMP-2:1.03±0.09 and 0.70±0.15,$p< 0.05$ *vs* Ang-II 1mM;MMP-9:3.37±0.14 and 2.20±0.10,$p< 0.0005$ *vs* basal);the effect of Ang-II on TIMP-1 was negligible.Some responses were potentiated by HG and FFA,being RSV able to partially revert them.MMP-2 and MMP-9 activities were coherent with their expression.RSV 1 and 5mM. upregulated MMP-2 activity (NG:+20±6% and +37±9%),with a less defined effect on MMP-9.Following this MMP-TIMP modulation,TGFβ increased after Ang-II (NG:from 217±19 to 337±24 and 391±21) and decreased after RSV (237±22 and 203±24 pg/ml/mg protein).Fibronectin and collagen-IV release showed a similar pattern in all the experimental conditions.

Conclusions: Ang-II induces pro-fibrotic responses in HMC,mainly *via* a dysregulation of the MMP-2/TIMP-2 pattern.These effects,amplified by HG and FFA,were partially reverted by RSV,suggesting a potential new therapeutic application of this HMGCoA-reductase-inhibitor.

Memory Stick

PRETREATMENT EFFECT OF *CORNUS MAS L*. ON ALLOXAN-INDUCED DIABETES IN RATS

Sedigheh Asgary[1], F. Shamsi[1], S. Kazemi[1], A. Adelneia[1], M. Rafieian[2]

[1]Cardiovascular Research Center/ Isfahan University of Medical Sciences, Isfahan, [2]Medicinal Plant Reserch Center Shahrekord, Shahrekord, Iran

Background: Diabetes mellitus is a chronic and major endocrine disorder. phytochemicals present in medicinal plant may play a preventive role in the development of diabetes and diabetes-related complication. (*Cornus mas*) is one of those promising plants. This study was designed to investigate the pretreatment effects of fruit of *cornus* in diabetic rats.

Methods: Diabetes mellitus was induced in 24 out of 32 male Wistar rats, using intraperitoneal (IP) injection of 120 mg/Kg BW Alloxan. The diabetic rats were divided into three groups; (I) diabetic rats, (II) diabetic rats treated with *cornus* (2g/daily), and (III) diabetic rats treated with Glibenclamide (0.6 mg/kg BW, daily). The remaining non-diabetic rats received neither alloxan nor the mentioned plants. 28 days before of induction of diabetes, total of groups were treated with ordinary diet except the second group, that received either diet include the fruit of the *cornus mas* (2g/daily). Diabetic rats were treated either with the cornus or glibenclamide daily for 4 weeks.

Result: Fasting blood sugar, decreased meaningfully in diabetic rats treated with plant and diabetic rats treated with glibenclamide ($P< 0.05$). Concurrent histological studies of the pancreas of these animals, demonstrated the same result. On the base of histologic result, *cornus mas* have a significant effect on increasing the size of pancreatic islets in diabetic rats.

Conclusion: The observations of this study indicate that antioxidant present in *cornus mas* can exert antihyperglycemic effect in alloxan-induced diabetic rats.

Keywords: *Cornus mas*, diabetes, alloxan, rat

Memory Stick

EFFICACY OF PERCUTANEOUS SHUNT CLOSURE FOR HEART SEPTUM DEFECTS

Tohid Emami Meybodi[1], M. Emami[2], M. Dehghani[3], M. Behjati[3]

[1]Cardiovascular Research Center, [2]Afshar Heart Center, [3]Yazd University of Medical Science, Yazd, Iran

Introduction: Surgical repair of cardiac defects (ASD and VSD) is a safe, widely accepted procedure with negligible mortality. However, it is associated with morbidity, discomfort and a thoracotomy scar. As an alternative to surgery, a variety of devices for transcatheter closure of these defects have been developed. The immediate and short-term results of theirs deployment have been reported frequently. Objective was to describe the midterm efficacy and safety of percutaneous closure.

Methods: 135 Occluder devices were used in 127 patients to close ASDs (116), VSD (11) between March 2002 and July 2008 Detailed data were collected at implantation and they have been followed clinically and investigated with electrocardiogram and echocardiogram at a mean of 48 (76 month to 1 week) months after implantation.

Results: The ages at implantation ranged from 7 to 70 years. Of few residual shunts noted early after implantation, no one persisted at latest follow-up. There were no new shunts. There were no new ECG abnormalities. There were no cases of frame fracture. There has been only one mortality (in VSD group). There were 5 device emboli in ASD and 2 in VSD devices. TIA is seen in one ASD closure 16 months after implantation. Hemolysis was detected in 2 VSD patients.finally,in statistic analysis result were acceptable (P.value< 0.05).

Conclusion: At midterm follow-up the percutaneous shunt closure is durable and effective, with an acceptable adverse sequel. So we can conclude that this procedure is safe and can help patient with these defects.

Keyword(s): VSD-ASD- septum defect-shunt

Memory Stick

ASSOCIATION BETWEEN FOOD INSECURITY, AND CVD AND ITS RISK FACTORS IN IRAN

Morteza Rafiei[1], H. Entezari[2], M. Boshtam[3]

[1]Medical Education Development Center/Isfahan University of Medical Sciences, [2]Nutrition, Health Center Faculty/Isfahan University of Medical Sciences, [3]Cardiovascular Research Center/Isfahan University of Medical Sciences, Isfahan, Iran

Objective: Researches report high obesity and other nutritional CVD risk factors prevalence as the first mortality cause in ran. Household food security is an index reflecting access of families to food. This study tries to investigate this index regarding food security and food insecurity and its different social classifications as well as the statue of CVD and their nutritional risk factors in each of these groups to define the association between economic aspect of food access and CVD.

Methods: At first a standardized scale for food security was made. Food security was investigated among 3000 families in Isfahan. The statistical approach chosen was the Racsh model, a concise one- factor non-linear Item Response Theory (IRT), a model that first obtained data using a specialized software estimating the measurement scale and computing an "item calibration" value for each question in the scale. The item calibration score indicates the relative severity of the food insecurity by each question as well as the whole questionnaire. In addition, the questions on CVD and their risk factor were asked from the samples.

Results: The findings of this study showed that although food consumption patterns and dietary quality are highly income - depended dietary choices, particularly in higher- income groups are also driven by non- economic forces. Also, there is direct correlation between food insecurity and CVD risk factors.

Conclusion: We conclude that food insecurity can cause of high prevalence of CVD risk factors in our society.

Memory Stick

ARTERIAL PULSE IMPACT ON BLOOD FLOW

Merab Beraia[1], G. Nebieridze[2]

[1]MRI, Institute of Clinical Medicine, [2]Medical Center, Health, Tbilisi, Georgia

Objective: At the present it is impossible to describe blood flow only by the Navier-Stokes equations because blood flow in the large arteries is unsteady, with the flow separation and waveform propagation of the viscoelastic mass. The purpose of this paper is to study impact of the arterial pulse on blood flow and initial factors of atherogenesis.

Methods: In 12 healthy man (25-39 years of age) mean, peak velocity, mean, net flow has been investigated by MR angiography.

Results: Initial velocity is registered after 43msec of the ECG-R wave, and it is differing from zero at all sites of the aorta, although net flow is equal to zero. Womersley number from the ascending to the abdominal aorta decreases from -13 to 5, flow is modified from inertial to viscous. In the aortic arch in protodiastole blood flow separated into opposite directed streams resulting to wave superposition with the high net flow. At the isthmus area antegrade directed waves interference. Here flow acceleration in protodiastole 6 times higher than that in systole. At the boundary reflection wave can shift the vessel wall.

Conclusions: Pulse wave increases stain rate to the contiguous flow layers. At the circular sites in protodiastola flow separation/recirculation and wave superposition promotes high net flow, but wave interference constrains the vessel wall to shift.

Memory Stick

MYTH OR FACT; IS THERE A ROLE FOR PERSONALITY IN ATHEROSCLEROTIC PLAQUE PROGRESSION? PRESENTING A PROSPECTIVE COHORT STUDY

Paula M.C. Mommersteeg[1,2], W.H. Aarnoudse[2], J. Denollet[1], J.W. Widdershoven[2]

[1]CoRPS, Tilburg University, [2]Cardiology, TweeSteden Hospital, Tilburg, The Netherlands

Objective: The present study aims to identify the risk of negative affectivity and social inhibition, components of the Type D personality type, in the progression of vascular irregularities and mild stenosis. We hypothesize that in addition to behavioral risk factors, biological mechanisms may mediate the relation between personality and cardiovascular disease progression.

This hypothesis is plausible as on one side a significant part of the variance in systemic IL6, CRP and fibrinogen, involved in the development of atherosclerotic disease, is due to shared genetic influences. On the other side negative affectivity as Type D personality and depression have been related to an overall increased pro-inflammatory cytokine pattern and a decreased anti-inflammatory pattern. Recent findings support the existence of a genetic profile related to pro-inflammatory cytokines in both healthy subjects and patients affected by mood disorders.

Methods: 500 patients with angiographically confirmed vascular irregularities will be included at the TweeSteden Hospital Tilburg. Baseline and one year measurements will include questionnaires on negative affect components as depression, exhaustion, hostility and Type D personality, as well as health status and angina complaints. Routine cardiovascular risk markers, and metabolic syndome components are measured. Blood samples are being collected for hsCRP, fibrinogen, DNA storage, and pro- and anti-inflammatory cytokine assessment. Two year follow-up health status, angina complaints and major adverse cardiac outcomes will serve as outcome variables.

Results: data collection has started in 2009. Preliminary findings will investigate the relation between routine cardiovascular risk factors, vascular irregularities and the negative affect markers.

Memory Stick

ASSOCIATION STUDY OF ABCB1 AND 5HT2C GENETIC POLYMORPHISMS AND METABOLIC SYNDROME IN FEMALE PATIENTS WITH SCHIZOPHRENIA

Tamara Božina[1], H. Ljubić[1], M. Rojnic Kuzman[2], N. Božina[2], J. Sertić[1]

[1]Zagreb University School of Medicine and University Hospital Zagreb, [2]University Hospital Zagreb, Zagreb, Croatia

Background: Second generation antipsychotic (SGAs) drugs have brought significant progress in treatment of schizophrenia. However, among patients who respond to treatment well, there is a significant number of those who develop metabolic syndrome (about 50%). Interindividual differences in responses to SGAs point out that genetic factor may be relevant. The objective of this study was to determine the association of genetic polymorphism of 5-HT2c and ABCB1 and metabolic abnormalities among female patients with DSM IV schizophrenia spectrum disorders treated with SGAs.

Methods: We recruited 121 female schizophrenic patients following DSM-IV criteria, who were acutely psychotic and treated with olanzapine or risperidone for up to 3 months. Metabolic syndrome developement (which included assessment of the increase of fasting glucose levels in blood, fasting total cholesterol, LDL, HDL and triglyceride levels, blood pressure and waist and hip circumferences and body mass index) was assessed. Genomic DNA was isolated from a whole blood sample of patients and exon 21 2677G>T/A and exon 26 3435C>T gene variants of ABCB1 were identified by Real time PCR method in Roche LightCyclerâ. -759C>T of 5-HT2c gene was analysed by PCR-RFLP method.

Results: We found a significant association of -759CT 5-HT2c and the increase of waist circumference, fasting glucose and triglycerides in blood after a 3-month period. 2677GT ABCB1 variants were significantly associated with the increase of fasting glucose in SGA-treated patients. Our data indicate a possible influence of -759CT 5-HT2c and ABCB1 2677GT genetic polymorphisms on the development of metabolic abnormalities among female patients treated with SGAs.

Memory Stick

BIPOLAR SUPERNUMERARY RENAL ARTERY

Abolhassan Shakeri Bavil

Tabriz University of Medical Sciences, Tabriz, Iran

Case report: The variations of renal arteries are considered critical issues that surgeons should have thorough envision and appreciation of the condition. Variations of these vessels may influences urological, renal transplantation and laparoscopic surgeries. We present a case of bilateral accessory renal artery with a striking pre-hilar branching pattern encountered upon conventional angiography for imaging of the renal arteries of a healthy 30-year-old man, renal transplant donor. The right kidney received two renal arteries from the aorta including a main hilar and one lower polar. However, the left accessory artery while originated from the aorta, simultaneously, supplied both upper and lower renal poles following its pre-hilar division that replaced upper/apical and lower segmental arteries of the single main renal artery, respectively. The left main renal artery divided into two anterior and posterior segmental arteries. Whether this should be categorized either as an accessory hilar artery or a unique variant of renal arterial supply, the so-called bipolar supernumerary renal artery, is a matter of debate. We discuss possible embryologic origin and clinical aspects of accessory renal artery.

Memory Stick

SINGLE NUCLEOTIDE POLYMORPHISMS AT THE ADIPONECTIN LOCUS AND RISK OF CORONARY ARTERY DISEASE IN A TUNISIAN POPULATION

I. Boumaiza[1], A. Omezzine[1], **Jihène Rejeb**[1], L. Rebhi[1], K. Kaouthar[1], N. Ben Rejeb[1], N. Nabli[1], A. Ben Abdelaziz[2], E. Boughzala[3], A. Bouslama[1]

[1]Biochemistry Department, UR MSP 28/04, [2]Information System Direction, [3]Cardiology Department, Sahloul University Hospital, Sousse, Tunisia

Background: Adiponectin is an adipocyte derived hormone and an essential modulator of insulin sensitivity. Several studies suggest an important role of adiponectin in the process leading to atherosclerosis, thus indicating the adiponectin gene as a potential candidate for coronary artery disease. Two single nucleotide polymorphisms (SNPs) at the adiponectin locus (+45 T/G and +276G/T) have been associated with low circulating adiponectin levels, Insulin resistance, and type 2 diabetes.

Aims: The objective was to examine the association of 2 SNPs (45 T/G and 276 G/T) with risk of coronary disease in a Tunisian population.

Methods: We have recruited 316 Tunisian patients, documented by coronary angiography. Significant Coronary Stenosis (SCS) was defined as a luminal narrowing of ≥ 50% in at least one major coronary artery. Genotyping was performed by PCR-RFLP. Lipids and apolipoproteins were measured.

Results: After adjustments for confounders parameters, OR of SCS associated with 276G/T mutated genotypes was 0.472 [0.195-0.842] p=0.046). Whereas the mutated genotypes at the +45 T/G polymorphism was significantly associated with increased SCS only in obese (OR=3.31 95% CI [0.996-11.05] p=0.049) versus (OR=1.71 95% CI [0.467-6.269] p=0.418) in non obese. A potential protective effect was also observed for the haplogenotypes TT/TT (OR= 0.548 [0.306-0.982] p= 0.043) in all the studied population.

Conclusion: Mutated genotypes at +276 G/T (TT+GT) and TT/TT haplogenotypes seems to reduce the risk of SCS. Whereas mutated genotypes at +45 T/G (GG+TG) was associated with an increase in SCS only in obese group.

Memory Stick

PROOXIDANT- ANTIOXIDANT BALANCE (PAB) WITH HEALTY PEOPLE AND PEOPLE WITH HIGH PHISICAL ACTIVITY

Tamara Gojkovic, V. Markovic, M. Kostic

University of Belgrade, Belgrade, Serbia

Introduction: In human body exists balance between prooxidants and antioxidants. Oxidative stress is defined as an imbalance between prooxidants and antioxidants in favor of prooxsidants. In this stady values of prooxidant-antioksidant balance was shown in healty people and people with high physical activity by using PAB assay.

The aim of study was determination PAB values in children, young persones, middleaged persones, elder persones and sportists, also to exame does PAB values have correlation with oxidative stress.

Materials and methods: Method is colorimetricis and it is based on reactions 3,3´,5,5´-tetramethylbenzidine and its cation with hydrogen peroxide in enzymatic reaction catalised by peroxidase enzyme and a chemical reaction with uric acid. As biological material we used plasma. PAB values are expressed in HKU

Results: Has been proofed that PAB values are higher in elder persones (371±153HKU) and phisicaly active persones-sportists (414±205HKU) but,lower in children (205±159HKU) in relation to the middleaged persones(344±144 HKU). A significant statistic differences were found for PAB values in different kind of sportists (from 301±93 to 662±150). Also there are differences between PAB values in difrenet sexes, in boys are higher than girls , but in women are higher than men. Significant statistic difference was esthablished between sportists and other respondents for parameters´s values of oxidative stress wich is increased in sportists.

Conclusion: This study showed that PAB values are in correlation with oxidative stress status and it can point at on prooxidant-antioxidant imbalance in healty persones with increased risc development of oxidative stress like sportists and elder persones.

Memory Stick

ZINC-GENE INTERACTIONS ON INFLAMMATION AND RISK TO DEVELOP CVD IN THE ELDERLY

Robertina Giacconi[1], S. Kanoni[2], P. Mecocci[3], M. Malavolta[1], G. Dedoussis[4], E. Mocchegiani[1]

[1]INRCA, Ancona, Italy, [2]Harokopio University, Athens, Greece, [3]University of Perugia, Perugia, [4]Harokopio University, Athens, Italy

Inflammation and genetics are prominent mechanisms in the pathogenesis of cardiovascular disease (CVD), while growing literature suggests zinc deficiency as a promoter of atherogenesis. Indeed, a chronic zinc impairment affects the antioxidant response determining an increased vulnerability to oxidative stress and inflammation. Genetic polymorphisms of genes involved in inflammation and zinc homeostasis have been implicated through a differential inflammatory response in the CVD susceptibility. However, the risk allelic variants for CVD frequently occur in the general population and only adequate gene-environment-polymorphism interactions will promote the disease development. For this purpose, two recent studies showed the impact of gene-nutrient-interactions on the inflammatory status of the elderly and in the risk to develop CVD, suggesting a crucial role for an adequate zinc intake in old people. One investigation demonstrated an association between −174GG Interleukin-6 (IL-6) and higher plasma IL-6 levels compared to GC/CC genotypes coupled with increased zinc diet score. The second study reported an association among +1245 Metallothionein-1A (MT) polymorphism and +647/+1245 MT-1A haplotype with the susceptibility to CVD in Greek population, but not in Italy. MT-1A CG haplotype modulated enzyme antioxidant activity in Greece (catalase and glutathione peroxidase) and in Italy (superoxide dismutase) and it was associated with decreased intracellular zinc release (iZnR) by MT in PBMCs from Greek patients. Despite the similar Mediterranean dietary pattern in these two countries, Greek elderly subjects showed lower zinc status, reduced zinc dietary intake and increased oxidative stress than Italian ones, suggesting a role of gene-diet-interaction in the CVD predisposition.

Memory Stick

THE RELATION BETWEEN PREGNANCY AND ACCUMULATION OF FAT AROUND HEART

Sina Naserian[1], S. Sheykh al Eslami[1], V. Khalili[1], S. Shamdani[2]

[1]Islamic Azad University Tehran Medical Branch, [2]Islamic Azad University Science and Research Branch, Tehran, Iran

Purpose: Accumulation of fat around heart is the first step of many heart problems and diseases which needs accurate survey to search for the causes and try to eliminate as far as possible.

The number of pregnancy in women in this case is an important parameter as it may help to salve the problem in an accurate way.

Therefore, a research was planed out to look for the causes of this problem among different patients and search the causes in this respect.

Method: Three years files of the patients referred to a hospital ,heart section, were reviewed and the patients were classified in different groups according to their sex, age and the number of their children ,etc.

Result: The result showed that the female subjects are more prone to this problem and those with more children , over three , are suffering more than others.

Conclusion: From the result of the present study, we can conclude that women with higher number of pregnancy among population are in higher risk of heart problems which will be discussed in detail in full paper.

Keywords: Pregnancy, sex and age

Memory Stick

LETTER OF MOTIVATION

Oluropo Ebun, Family Medicine

Globacom Nigeria Limited, Ibafo, Nigeria

The European Atherosclerosis Society is a organisation that has come to endear itselff in the heart of many. Over the years i have been following the activi ties of this society and havegone through some of the lecstures delivered in the conferences organised by them.

Atherosclerosis itself is a field that can not be ignored in the 21th century considering the fact that everyone is concerned about the eco system of our world,which is deterinating as a result of industrialisation of our world.

Industrialisation being good in itself can however be done in a way that it does not affect the eco system and the knowledge of this can mainly come from the type of conferences being organised by European Atherosclerosis Society.

For a longtime i have been follwing the the European Atherosclerosis Society and when this opportunity came up it was just to fascinating for me to let it slip by.

The desire to see my world a greener and better place to live in is a very strong motivation for meto join the society.I believe i can contribute positively to the society as a participant and live the world a better place than i met it for future generations to live in.

Memory Stick

STUDY OF THE PREDISPOSAL RISK FACTORS IN PATIENTS WITH ACUTE CORONARY SYNDROME IN MACEDONIA

S. Patiakas[1], **Charalampos Charalampous**[2]

[1]Microbiological Laboratory of General Hospital of Kastoria, [2]General Hospital of Kastoria, Kastoria, Greece

Aim: To register and study the predisposal epidemiologic factors for the development of Acute Coronary Syndrome (ACS), considering that they also play an important role in its further prognosis.

Material & Method: In the study participated 143 patients (87 men and 56 women) with the average age of 72,4 years. 39 of them (27,3%) had unstable angina, 44 (30,8%) non-ST segment elevation myocardial infarction (NSTEMI) and 60 (41,9%) ST segment elevation myocardial infarction (STEMI).

Results: The several epidemiologic factors were the following as you can see at the table 1.

Conclusions: It is, therefore, shown by our study, that: 1) The most important risk factor for patients with ACS is smoking. 2) In patients with unstable angina and non-ST segment elevation myocardial infarction, the arterial blood pressure and hypercholesterolemia follow, while in patients with ST segment elevation myocardial infarction, diabetes mellitus and hypertension follow. 3) In many patients coexist more than one predisposal factors, and finally 4) hypercholesterolemia and obesity play an important role in the evolution of the ACS.Taking into account that all the previous ACS predisposal factors -except for heredity- are changeable, one can realize our great responsibility for their prevention.

Memory Stick

EFFECTS OF THE TREATMENT OF DMA (6 ,7-DIMETHOXI-4-N-(3`-N`,N`-DIMETHYL)PHENILAMINOQUINAZOLINE) ATHEROSCLEROSIS IN MICE KO LDLR-/ -

Thais Franchini Tornatore, S.A. Rocco, O.R. Coelho, K.G. Franchini

UNICAMP, Campinas, Brazil

Atherosclerosis is characterized by the recruitment of monocytes and lymphocytes to the artery wall. A triggering event for this process is the accumulation of minimmally oxidized LDL, which stimulates the overlying endothelial cells to produce a number of pro-inflammatory molecules, including hemodynamic forces, sex hormones and infection.

We demonstrated that DMA,6 ,7-Dimethoxi-4-N-(3`-N`,N`-dimethyl phenilaminoquinazoline, have an anti-atherogenic effect in mice LDLr-/-. This effect could have a relationship with an anti-inflammatory property of adenosine in tissues, by the inhibition of adenosine kinase.

Results showed that DMA administrated by oral via, as prevent treatment, as pre-established lesions, in mice LDLr-/- with hypercholesterolemic diet (1,25% cholesterol and 0,5% colic acid), decreases atherosclerotic lesions in 67% and 52% respectively in relation of the vehicle group. These data was obtained by planimmetric study of atherosclerotic lesions in the base of aortic arthery with Oil Red O especific for lipids. The hypercholesterolemic diet by 15 and 30 days, decreases serum tryglicerides in 50%, in the other way, the treatment with DMA cancelled this reduction. At the same time, HC diet by 15 and 30 days increases total cholesterol serum, in 2 times in mice LDLr-/-. Mice treatment with DMA and hypercholesterolemic diet showed serum cholesterol higher than the HC group (control). Results demonstrated that this increase have relationship with higher HDL serum level in mice treated with compound. All mice in treatment (DMA and Vehicle group) had the weight decreased.

Memory Stick

EFFECTS OF SOY ISOFLAVONE EXTRACT SUPPLEMENTS ON BLOOD PRESSURE IN ADULT HUMANS: SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED PLACEBO-CONTROLLED TRIALS

Kyoko Taku[1], N. Lin[1,2], D. Cai[2], J. Hu[3], X. Zhao[3], Y. Zhang[3], P. Wang[4], M.K. Melby[5], L. Hooper[6], M.S. Kurzer[7], S. Mizuno[8], Y. Ishim[9], S. Watanabe[5]

[1]Informaton Center, Nitional Institute of Health and Nutrition, Tokyo, Japan, [2]Department of Clinical Nutrition, Changhai Hospital, Second Military Medical University, Shanghai, [3]Department of Nutrition and Food Hygiene, [4]Department of Social Medicine and Health Education, School of Public Health, Peking University, Peking, China, [5]Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan, [6]School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK, [7]Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA, [8]Center for Collaboration and Partnership, [9]Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo, Japan

Objective: Reported effects of different soy products on blood pressure vary. This systematic review and meta-analysis was performed to clarify the effects of soy isoflavone extract supplements on systolic and diastolic blood pressure in adult humans.

Methods: PubMed, CENTRAL, ICHUSHI, and CNKI were searched in June 2009 for relevant randomized placebo-controlled trials (RCTs). Study data and indicators of methodological validity were independently extracted by 2 authors using predefined data fields. Meta-analysis was carried out in Review Manager 5.0.22.

Results: Searches identified 3740 articles, of which 14 RCTs (789 participants) were included. Daily ingestion of 25-375 mg soy isoflavones (aglycone equivalents) for 2-24 weeks significantly decreased systolic blood pressure by 1.92 mmHg (95% CI: −3.45 to −0.39; P = 0.01) compared with placebo (heterogeneity: P = 0.39; fixed effect model), in adults with normal blood pressure and prehypertension. The effect was not lost on sensitivity analysis. Subgroup analyses suggest greater effects in studies longer than 3 months, in Western populations, at lower doses, and in studies at lower risk of bias. Soy isoflavones did not affect diastolic blood pressure (−0.13 [95% CI: −1.03 to 0.78] mmHg, P = 0.78; heterogeneity: P = 0.20, fixed effect model).

Conclusions: Soy isoflavone extracts significantly decreased systolic blood pressure but not diastolic blood pressure in adult humans, and no dose response relationship was observed. Further studies are needed to address factors related to the observed effects of soy isoflavones on systolic blood pressure and to verify the effect in hypertensive subjects.

Memory Stick

GENERAL AND SPECIFIC QUALITY OF LIFE FOR THE PATIENTS WITH ISCHEMIC HEART DISEASE REFERRING TO MEDICAL CENTERS OF KASHAN 2007

Mohsen Taghadosi[1,2]

[1]Kaushn Medical Science, [2]Meical Surgical Nursing, Kaushan, Iran

Background: Ischemic heart diseases are becoming the most common and main causes of heart diseases and mortalities all over the world affecting quality of life of the patients. Impaired quality of life associated with ischemic heart disease is the most challenging problem facing the patients and the community burdened with excessive costs.

Materials: They were conscious and inclined to answer the questions, and were not hospitalized ever since 1-2 months before. General and specific qualities of life of the clients were measured respectively by means of SF-36 and SAQ instruments and then scored as bad, median, good, and very good based on the test scores.

Results: The findings showed that the mean age of the clients was 60/5±12/5 years. Mean of life quality for patients with IHD was mainly good and very good based on the SAQ criterion, while it was good and median according to the SF-36 criterion. None of the patients had poor quality of life. Quality of life based on the SF-36 and SAQ criteria were viewed with a mean of 112/5±24 and 59/8±9/14, respectively. Factors affecting quality of life included age, place of residence (city or village), number of children, education, marital status, and sex. .

C**onclusions:** Increased number of underlying risk factors for heart disease, increased number of children, and the population's getting older were all associated with the meaningful low quality of life in the patients.

Memory Stick

INFLAMMATION ACTIVITY OF PERICARDIAL FAT IS INCREASED IN PATIENT WITH CORONARY ARTERY DISEASE: F-18 FDG TOF PET/CT STUDY

Sungeun Kim[1], E.J. Kim[2], J.W. Kim[2], H.S. Seo[2]

[1]Korae university Guro Hospital, [2]Cardiovascular Center, Korea University Guro Hospital, Seoul, Republic of Korea

Purpose: There is increasing evidence that Plaque inflammation could be assessed by F-18 FDG PET. Pericardial fat (PF) thickness is reported to be associated with coronary atherosclerosis. To our knowledge, no other study has measured pericardial fat (PF) inflammation or investigates its association with coronary artery disease (CAD). We evaluated investigated whether PF F-18 FDG (FDG) activity could be related to CAD using time of flight (TOF) PET/CT scanner.

Methods: In all 73 patients (39 with CAD (male 27, 52 7.9 yrs), 34 normal coronary (male 18, 53.8 9.3)) who underwent coronary angiography (CAG) were randomly studied. The TOF PET/CT images (Philips GEMINI TF) was performed within 1 week after coronary angiography (CAG).

Result: The FDG uptake of PF was measured using volumetric analysis tool of the PET/CT fusion image on a dedicated workstation. The PF was measured by manual tracing of a region of interest (ROI) at the pericardium on axial CT slices and then the maximum standardized uptake value (max SUV) in the ROI was calculated. Age and gender-adjusted max SUV of FDG on TOF PET/CT was significantly higher in the PF of patients with CAD than those of patients without CAD (mean 1.21 0.59 (to 0.62 to vs 1.8) vs 0.27 0.12 (0.15 to 0.39), P

Conclusion: Patients presenting with CAD have increased inflammation activity in the pericardial. Our findings might be contributed to understanding a linkage between pericardial fat and coronary artery disease.

Memory Stick

DEMONSTRATION OF CAROTID PLQUE INFLAMMATION BY F-18 FDG PET IN PATIENTS WITH ACUTE CORONARY SYNDROME

Sungeun Kim[1], E.J. Kim[2], J.W. Kim[2], H.S. Seo[2]

[1]Korea University Guro Hospital, [2]Cardiovascular Center, Korea University Guro Hospital, Seoul, Republic of Korea

Background: A systematic plaque instability is suggested in patients with acute coronary syndrome. Plaque inflammation could be assessed by F-18 fluorodeoxyglucose Positron Emission Tomography (18F-FDG PET). We investigated whether carotid plaque inflammation could be related to coronary plaque instability using 18F-FDG PET.

Methods: In 50 (male 14, 48.17.7 yrs) patients who were newly diagnosed as acute coronary syndrome (28 patients, male 6, 46.87.9 yrs) or stable angina (22 patients, male 13, 49.59.8), the co-registration of PET and contrast enhanced computed tomography (CT) images was performed within 1 week after percutaneous coronary intervention. Tthe multislice CT angiogram were acquired at 180 min on the Philips GEMINI TF scanner with 16 slice CT. The maximum standardized uptake values (SUVs) were measured in individual plaques.

Results: In all patients, carotid plaque with increased 18F-FDG uptake was observed in the fused PET/CT images. Age and gender-adjusted SUV of FDG on PET scan was significantly higher in the carotid plaques of patients with acute coronary syndrome than those of patients with stable angina (mean 4.131.24 (3.19 to 5.27) vs. 2.870.98 (2.47 to 3.62), p=0.003). There were no differences of risk factors between two groups.

Conclusions: The patients presenting with acute coronary syndrome demonstrate simultaneous increase of inflammatory activity of the carotid plaque, supporting a potential causal role of inflammation regarding widespread plaque destabilization associated with acute coronary syndrome.

Memory Stick

AORTIC STIFFNESS IN BEHCET´S DISEASE

T. Aksu, E. Guler, **Kumral Cagli**

Turkiye Yuksek Ihtisas Hospital, Ankara, Turkey

Introduction: Behcet disease (BD) is a multisystemic disorder of unknown etiology. The incidence and nature of cardiac involvement in BD are not clearly documented yet. Aortic stiffness is reported as an independent risk factor for cardiovascular disease, requiring treatment.

Aim: The aim of this study was to assess aortic stiffness in BD.

Methods: A total of 54 patients with BD (mean age; 29,8±8.1 years) and 36 age and sex-matched controls (mean age; 28.1±4.7 years) were included. Aortic stiffness was evaluated by aortic strain and distensibility.

Results: Baseline characteristics were similar between groups. Dimension of cardiac chambers, aortic root dimension, valvular abnormalities, and conventional systolic function indices were similar in both groups. Ascending aorta aneurysm was not detected in any groups.

Aortic strain in patients with BD was found to be significantly less than in the controls (8.3±4.9% and 15.7±2.7% respectively,$p< 0.001$). Aortic distensibility was also significantly low in patients with BD when compared to controls (0.45±0.28 and 0.78±0.13 respectively, $p< 0.001$). Beta index values were significantly high in Behçet's patients (7.23±5.93 and 2.69±0.55 respectively, $p< 0.001$). This findings was consistent with increased aortic stiffness in BD.

Conclusion: We detected an increase in aortic stiffness, suggesting an inflammatory involvement of proximal aorta.

Memory Stick

PREMATURE TREATMENT EFECTS OF METABOLIC SYNDROME AS THE PREDICTOR OF DIABETES MELLITUS TYPE 2

Tea Gamezardashvili, N. Kipshidze

Department of Ischemic Heart Disease, National Center of Therapy, Tbilisi, Georgia

Insulin resistance and hyperglycemia are very frequent disorders. They are describing as the dominant risk factors for Diabetes Mellitus type 2 and cardiovascular diseases. We investigated 188 individuals with 3 or more risk factors of Metabolic Syndrome (MS). MS was defined according to the IDF criteria. They consider themselves practically healthy before the investigation. After having performed OGTT, impaired glucose regulation (IGR) was observed in 38.3% and already existing asymptomatic type 2 diabetes was observed in 14.9%. 72 patients were randomly assigned and divided into two groups. The first group patients were given recommendations about life style modification;The second group patients were administered the following medicines: ACE inhibitor, statin (Atorvastatin 20mg, Simvastatin 20mg or Rosuvastatin 10mg) and Metformine (1000 mg/day); also were given recommendations about life style modification. OGTT was once more performed in both groups after six months. After 6 months: In the first group in 8 patients (22.2%) glucose metabolism regulated, in 6 patients (16.7%) developed type 2 diabetes and the condition of 22 patients (61.1%) didn't change. In the second group In 17 patients (47.2%) glucose metabolism regulated, only in 3 patients (8.3%) developed type 2 diabetes and the condition of 16 patients (44.5%) didn't change.

Conclusions: Metabolic Syndrome as the cluster of Cardio Vascular Diseases risk factors is the predictor of type 2 diabetes; early therapy with ACE inhibitor, statin and Metformine contributes the discontinuation of the above mentioned pathologic processes and prevents the development of type 2 diabetes.

Memory Stick

INFLUENCE OF CETP, PPARA, APOE AND APOAI POLYMORPHISMS ON HDL-C, APOAI, LPAI AND LPAI:AII CONCENTRATIONS: THE PRIME STUDY

Quang Do[1,2], H. Nazih[2], G. Luc[3], D. Arveiler[4], J. Ferrières[5], A. Evans[6], P. Amouyel[7], F. Cambien[8], P. Ducimetière[9], J.-M. Bard[2], PRIME Study

[1]Biochemistry, Hanoi University of Pharmacy, Hanoi, Vietnam, [2]Laboratoire de Biochimie et Laboratoire d'Etude du Polymorphisme de l'ADN, University of Nantes - Faculty of Pharmacy, Nantes, [3]Institut pasteur de Lille, Inserm U545, Lille, [4]Centre MONICA, Faculté de Médecine, Université Louis Pasteur, Strasbourg, [5]Centre MONICA, INSERM U558, Faculté de Médecine, Toulouse, France, [6]Department of Epidemiology and Public Health, Queens University, Belfast, UK, [7]Centre MONICA, INSERM U744, Institut Pasteur, Lille, [8]INSERM U525, Faculté de Médecine Pitié Salpêtrière, Paris, [9]INSERM U780, Hôpital Paul Brousse, Villejuif, France

Introduction: The plasma level of HDL-Cholesterol (HDL-C) is known to be inversely associated with cardiovascular risk. However, besides lifestyle, gene polymorphism may influence the HDL-C concentration.

Objective: The aim of this study was to investigate the possibility of interactions between CETP, PPARA, APOE and APOAI polymorphisms and HDL-C, apoAI, LpAI and LpAI:AII in a sample selected from the PRIME study population who remained free of cardiovascular events over five years' follow-up.

Methods: Healthy individuals (857) were randomly selected for genotyping the PRIME Study subjects. The population was selected so as to provide 25% of subjects in the lowest tertile of HDL-C (≤28 mg/dL) in the whole PRIME Study sample, 25% of subjects in the highest tertile of HDL-C (≥73 mg/dL) and 50% of subjects in the medium tertile of HDL-C (28-73 mg/dL). Genotypes were determined by TaqMan based allelic discrimination method

Results: The CETP A373P rare allele *c*, was less frequent in the group of subjects with high HDL-C, apoAI, LpAI and LpAIAII concentrations. ApoAI and LpAI were also found higher in the presence of the ε2 allele coding for APOE. The effect of the CETP A373P rare allele *c* on HDL-C was independent of all tested parameters except triglycerides.

Conclusions: The respective effect of these polymorphisms and triglycerides on cardiovascular risk should be evaluated prospectively.

Memory Stick

UNUSUAL STEROLIC MIXTURE, AND 24-ISOPROPYLCHOLESTEROL, FROM THE SPONGE *CIOCALYPTA* SP. REDUCE CHOLESTEROL UPTAKE AND BASOLATERAL SECRETION IN CACO-2 CELLS

Quang Do[1,2], L. Landeghem[3], G. Wielgosz-Collin[4], M. Takoudju[5], J.-M. Huvelin[6], J.-M. Kornprobst[7], J.-M. Bard[6], G. Barnathan[4], H. Nazih[8]

[1]Biochemistry, Hanoi University of Pharmacy, Hanoi, Vietnam, [2]Biochemistry, [3]Inserm 915, University of Nantes - Faculty of Pharmacy, [4]Laboratoire de Chimie marine, Université de Nantes - Pôle Mer et Littoral, [5]Pôle Mer et Littoral, Université de Nantes, Nantes, [6]Laboratoire de Biochimie pharmaceutique, University of Nantes - Faculty of Pharmacy, [7]Laboratoire de Chimie marine, Université de Nantes-Pôle Mer et Littoral, nantes, [8]Laboratoire de Biochimie pharmaceutique, University of Nantes - Faculty of Pharmacy, Nantes, France

Introduction: Phytosterols are naturally occurring sterols in the plant equivalent of mammalian cholesterol. These major plant sterols are absorbed to a much lesser extent than cholesterol and reduce cholesterol absorption.

Objectives: The goal of the present study was to determine whether a sterolic mixture and its major component 24-isopropylcholesterol isolated from a marine sponge, *Ciocalypta* sp., affect the cholesterol uptake and secretion by differentiated Caco2 cells.

Methods: Caco2 cells were incubated in a mixed micellar solution in the presence of [1,2-^{3}H(N)]-Cholesterol in DMEM. Sterolic mixture or 24-isopropylcholesterol were added on the apical side of the membrane. After 24h of incubation, radioactivity was measured in the basolateral media.

Total RNA was isolated from Caco2 cells. The cDNA was then used for RT-PCR. The values were normalized using 6S rRNA as an endogenous internal standard.

Results: Sterolic mixture and 24-isopropylcholesterol in the apical medium induced a reduction of ^{3}H-cholesterol secretion into basolateral compartment and reduced significantly ^{3}H-cholesterol uptake and enhanced the expression of ABCA1.

b-sitosterol also reduced cholesterol uptake and secretion (42% and 29%, $p < 0.05$) but this effect was lower than for 24-isopropylcholesterol.

Conclusion: The study shows for the first time that a sterolic mixture from a sponge *Ciocalypta* sp., and its major component, 24-isopropylcholesterol, decrease the uptake and secretion of cholesterol in Caco2 cells. Moreover, these sterols upregulate ABCA1 gene expression. Thus, these data add a new sterol, 24-isopropylcholesterol to sterols that reduce cholesterol absorption.

Memory Stick

EXTREMELY HIGH PREVALENCE OF METABOLIC SYNDROME AMONG ARAB YOUTH: A CALL FOR EARLY INTERVENTION

Nasser Al-Daghri, RIYADH Cohort

King Saud University, Riyadh, Saudi Arabia

Background: Epidemiologic studies have suggested an increased prevalence of metabolic syndrome (MS) among adults in the Middle-East. This study aims to determine the prevalence of MS in a large cohort of Saudi children and adolescents.

Materials and Methods: In this cross-sectional observational study, a total of 1,231 randomly selected Saudi children and adolescents aged 10-18 years were recruited. Subjects´ information was generated from a database of more than 10,000 Saudi citizens from the existing Biomarkers Screening in Riyadh Program (RIYADH Cohort), Saudi Arabia. Anthropometrics included body mass index (BMI), blood pressure, as well as waist and hip circumferences. Fasting blood glucose and lipid profile were determined using routine laboratory procedures. The modified definition of ATP-III (NHANES III) was used for the diagnosis of MS.

Results: The overall prevalence of complete MS was 9.4 % [Confidence-Interval (CI) 7.8-11.0]. Age-adjusted prevalence according to the European standard population is 5.8 %. Boys had a higher prevalence than girls [10.3 % (CI 8.2-12.4) versus 8.1 % (CI 5.7-10.5)]. Low HDL-cholesterol was the most prevalent of all MS risk factors, affecting 86 % (CI 85.0-88.6) and hypertriglyceridemia the second most prevalent, affecting 33 % (CI 30.6-35.8) of the subjects.

Conclusion: The prevalence of MS manifestations among Arab children is extremely high, with dyslipidemia being the most common MS abnormality. Screening for dyslipidemia among Saudi children is warranted especially among those most at risk. Scientific inquiry into the molecular causes of these manifestations should be pursued as a first step in the discovery of etiologic therapies.

Memory Stick

BOSENTAN ATTENUATES DIABETIC CARDIAC COMPLICATIONS IN MICE WITHOUT AFFECTING BLOOD GLUCOSE

Bo Yang, Y.D. Chen, T.D. Li

Cardiology Department, Chinese PLA General Hospital, Beijing, China

Aims: To investigate the role of ET-1 in diabetic cardiac complication and the potential protective effect of Bosentan.

Methods: Male C57BL/6 mice with 6-week old were divided into 2 groups (N=10) : DM group (diabetes group) and DM-B group (diabetes with Bosentan group). STZ was injected as 200 mg/Kg for single dose, i.p. Fasting blood glucose (FBG) was measured at 0- , 1- , 2-week after STZ injection. Bosentan (100mg/Kg) and placebo was given i.g. once a day.

Results: After eight weeks of diabetes, FBG was similar between groups (DM mice 535.1±19.1 vs. DM-B mice 516.7±23.5 mg/dl, P=0.0707). After six months of diabetes, mice in DM-B group remained lower SBP than DM group (128.5±1.2 vs. 130.3±1.3 mmHg, P=0.0048). Electron microscopy study revealed a disruption of sarcomere and myofibril structure in heart of DM mice, which is partially prevented in DM-B mice. Furthermore, area of interstitial fibrosis is lower in DM-B mice. This lower area of interstitial fibrosis is associated with higher capillary density as measured by CD-31 immunostaining and much higher expression of cardiac VEGF mRNA in DM-B mice. The heart of DM-B mice also showed lower expression of fibrotic genes (TGF-ß, CTGF and Collagen-1). Furthermore, cardiac systolic function (ejection fraction, EF) of all mice decreased after 8 months of diabetes. However, the EF decrease is significantly attenuated in DM-B mice (46.7±1.8 vs. 40.3±2.5%, P< 0.001).

Conclusions: These findings indicate that ET-1 plays an important role in mediating diabetic cardiac complications. Bosentan attenuates diabetic cardiac complications without affecting blood glucose.

Memory Stick

ENDOTHELIN-1 RECEPTOR BLOCKADE INDUCED UP-REGULATING OF RAAS EXPRESSION IN TERMS OF BLOOD PRESSURE REGULATION

Bo Yang, Y.D. Chen, T.D. Li

Cardiology Department, Chinese PLA General Hospital, Beijing, China

Aim: To compare the expression level of RAAS in C57BL/6 mice with or without endothelin-1 receptor antagonist bosentan and the potential value in blood pressure regulation.

Methods and results: Bosentan (10 mg/Kg/d) and placebo were given to two groups of male C57BL/6 mice (N=5) seperately from 6- to 12-week old. After that, mRNA of liver, kidney and lung was isolated. Northern blot analysis demonstrated that the expression levels of AGT (angiotensinogen) in liver (P=0.0126), renin in kidney (P=0.002), ACE (angiotensin-converting enzyme) in lung (P=0.0041) were up-regulated in mice with bosentan. Another 15 male C57BL/6 mice were divided into 3 groups (N=5): mice in group A were given AT1 blocker valsartan (10 mg/Kg/d), mice in group B were given bosentan (10 mg/Kg/d), mice in group C were given both valsartan and bosentan (10 mg/Kg/d respectively). All mice admitted drug from 6- to 12-week old. No SBP (systolic blood pressure) difference can be found between groups before drug admitting. Six weeks after mono-therapy with valsartan, SBP was a little lowered (126.3±2.1 vs 121.9±3.5mmHg, P＝0.0425). Mono-therapy with bosentan little effect SBP (125.9±2.5 vs 122.3±3.2mmHg, P＝0.0827). While dual blockade with valsarvan and bosentan significantly lowered SBP (126.8±2.7 vs 102.6±2.8mmHg, P< 0.001).

Conclusion: We conclude that RAAS components will be up-regulating under ET (endothelin) blockade situation. Dual blockade of RAAS and ET system is benefit for blood pressure control.

Memory Stick

FENOFIBRATE TREATMENT REDUCES THE OCCURRENCE OF RECURRENT CARDIOVASCULAR EVENTS IN PATIENTS WITH TYPE 2 DIABETES

Christelle Foucher[1], K. Le Malicot[1], J.-C. Ansquer[1], V. Gebski[2], A. Keech[2]

[1]Laboratoires Fournier SA, Daix, France, [2]National Health and Medical Research Council Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

Objective: The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study evaluated the effects of fenofibrate among 9795 patients aged 50-75 years with type 2 diabetes mellitus in a randomized placebo-controlled trial over an average of five years of follow-up. We evaluated the effect of fenofibrate on recurrent cardiovascular (CVD) events (CVD death, non fatal myocardial infarction, non fatal stroke, coronary and carotid revascularization).

Methods: Wei, Lin and Weissfeld (WLW) method was used in this post-hoc analysis to estimate hazard ratios for first, second and third CVD events. This method considered all individuals to be at risk, irrespective of any previous events.

Results: During the study follow-up, 1295 patients had a first CVD event, with 317 and 72 developing second and third events, respectively. The numbers of fourth and fifth events were low (18 and 3, respectively). A significant overall treatment effect was observed on the first, second and third events (HR [95%CI): 0.868 [0.771 - 0.978], p = 0.020). In patient treated with fenofibrate, the numbers of first and second CVD events were significantly decreased compared to placebo (Table 1), with a greater treatment benefit for the second ones.

	Placebo (N=4900)	Fenofibrate (N=4895)	HR (95%CI) WLW	P-value
First	683	612	0.889 (0.797 - 0.992)	0.035
Second	181	136	0.751 (0.602 - 0.939)	0.012
Third	35	37	1.063 (0.669 - 1.687)	0.797

[Table 1: Treatment Effect on CVD Events]

Conclusion: Over 5 years**,** fenofibrate treatment significantly decreased the risk of recurrent cardiovascular events as compared to placebo.

Memory Stick

SITTING, STANDING, WALKING OR CLIMBING STAIRS AT WORK AND 11-YEAR PROGRESSION OF CAROTID ATHEROSCLEROSIS IN MEN WITH AND WITHOUT CVD

Niklas Krause[1], R. Brand[2], G.A. Kaplan[3], S. Kurl[4], J. Kauhanen[4]

[1]Medicine, University of California San Francisco, Richmond, [2]Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, [3]Epidemiology, University of Michigan, Ann Arbor, MI, USA, [4]Public Health, University of Eastern Finland, Kuopio, Finland

Background: Inconsistent literature regarding cardiovascular effects of occupational physical activity. Positive associations with progression of atherosclerosis were reported for high amounts of standing, energy expenditure, and relative aerobic strain, especially in those with pre-existing cardiovascular disease (CVD) (Krause et al. 2000, 2009).

Methods: Different work postures were assessed at baseline, 4- and 11-year relative to total work time. Effects on 11-year change of intima media thickness (IMT), measured by ultrasound in both carotid arteries of 621 working men in the Kuopio Ischemic Heart Disease Risk Factor Study, were estimated by multivariate regression analyses adjusted for baseline IMT, sonographer, and possible confounders including traditional risk factors plus conditioning leisure time physical activity, cardiorespiratory fitness, and income. Analyses were stratified by baseline IHD (defined by ECG, questionnaire, and medication history) or carotid artery stenosis (defined as at least 20% lumen reduction).

Results: Positive associations with IMT change were found for time in upright postures ($p=0.004$), walking on uneven ground ($p=0.016$), and climbing stairs ($p=0.002$). Interactions existed between IHD and these work postures ($p< 0.05$). Men working always upright and with IHD (n=79) experienced a 72% (95% CI 45-105%) change in maximum IMT compared to 42% (95% CI 24-34%) in men without IHD (n=542). Similar differences were observed for carotid artery stenosis. No significant associations were found for sitting, standing, or walking on even ground.

Conclusion: Dynamic upright work postures are positively associated with accelerated progression of carotid atherosclerosis, especially in men with CVD. Implications for CVD prevention and occupational rehabilitation deserve consideration.

Memory Stick

HEMORHEOLOGY AND HEMOSTASIS SYSTEM IN PATIENTS WITH CHRONIC CEREBROVASCULAR DISEASES AGAINST THE METABOLIC SYNDROME BACKGROUND

Sergey Orlov, N. Omelchenko, M. Tanashyan, V. Ionova

Research Center of Neurology, Moscow, Russia

Objective: To investigate the specifics of hemorheology and the hemostasis system in patients affected by chronic cerebrovascular diseases (CCBVD) against the background of metabolic syndrome (MS).

Materials and methods: 46 patients suffering from CCBVD were examined of whom 23 patients had the diagnosed MS. Basic hemostasis and hemorheology factors were investigated including erythrocyte aggregation, fibrinogen, von Willebrandt factor.

Results: The erythrocyte aggregation proved to be significantly higher in the MS patients, which was demonstrated by a higher level of the aggregation index reflecting the aggregate formation rate. For the MS patients it was 54[43.9;62.9]%, as compared with 45.8[31.9;52.9] for the non-MS patients ($p< 0.05$). The aggregation amplitude that characterizes the final aggregate size did not exceed normal values in both patient groups; however, this indicator was higher in case of the MS than without the MS - 8.5[7.2;10.3] and 7.8[7.1;8.7] arbitrary units, respectively. The fibrinogen level in the two groups was within the normal limits but it was significantly higher in the MS patients ($p< 0.01$), namely 3.9[3.4;4.7] g/l, as compared with that of the non-MS patients where it was 3.3[3.1;3.6]g/l. A significant increase of the antibody level against the von Willebrandt factor was revealed in the MS patients - 214[161;272]% - , as compared with the non-MS patients where it was 147[131;239]% ($p< 0.05$).

Conclusion: The MS contributes to formation of the protrombogenic status of hemorheology and hemostasis in CCBVD-affected patients, which can impair blood microcirculation in the brain thereby provoking the growth of neurological symptomatology.

Memory Stick

USING IMAGEJ FOR THE QUANTITATIVE ANALYSIS OF FLOW-BASED ADHESION ASSAYS IN REAL-TIME UNDER PHYSIOLOGIC FLOW CONDITIONS

Sascha Meyer dos Santos[1], U. Klinkhardt[1], R. Schneppenheim[2], S. Harder[1]

[1]Department of Clinical Pharmacology, University Hospital Frankfurt, Frankfurt, [2]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

A simple and robust method is presented for analysing raw videomicroscopic data of flow-based adhesion assays using the freely available public domain software ImageJ. The depicted procedures were exemplified by analysing platelet interaction with immobilised von Wille-brand factor and fibrinogen in flowing blood under physiological wall shear rates. Platelets adhered to vWf in a shear-dependent manner (adherent platelets ± SEM: 80 ± 4 at 150 s-1 wall shear rate, 233 ± 11 at 600 s-1 and 441 ± 15 at 1800 s-1) and neutralising GPIbα function abol-ished firm platelet adhesion. Furthermore, the described procedures enable the researcher to quantify the number of translocating platelets. The number of platelets translocating on vWf was likewise dependent on the applied wall shear rate (24 ± 5 at 150 s-1, 318 ± 24 at 600 s-1 and 1142 ± 89 at 1800 s-1). The suitability of the method was further demonstrated by the studying the adhesion to fibrinogen at 150 s-1. Platelet pre-incubation with 100 nM Abcixi-mab, 10 µM Tirofiban or 100 µM Eptifibatide dropped GPIIb/IIIa-dependent stable platelet deposition on fibrinogen to baseline level. The presented method to analyse videomicroscopic recordings from flow-based adhesion assays offers the advantage to provide a simple and reli-able way to quantify flow-based adhesion assays and provides the researcher a tool to rapidly gain quantitative data for statistical analyses. It can easily be applied to study adhesion mechanisms of cells in non-fluorescent modes without the need to deviate from the presented protocol.

Memory Stick

PHENOTYPIC DIFFERENCES OF HUMAN NEUTROPHILS OF CARRIERS OF THE PSGL-1 A AND B-ALLEL IN BINDING TO P-SELECTIN UNDER FLOW CONDITIONS

Sascha Meyer dos Santos, U. Klinkhardt, K. Lang, J. Parisius, K. Kuczka, S. Harder

Department of Clinical Pharmacology, University Hospital Frankfurt, Frankfurt, Germany

P-selectin glycoprotein ligand-1 (PSGL-1) interacts with selectins expressed on endothelial cells and supports the trafficking of leukocytes into inflamed tissues. PSGL-1 extracellular mucin domain contains decameric repeats that display genetic polymorphisms in the variable number of tandem repeats (VNTRs). The wildtype consists of 16 decameric repeats (designate A isoforms) and variants with 15 (B allele) and 14 (C allele) repeats that are possibly associated with reduced risk of vascular disease. We investigated the potential different adhesive capacity of these natural variants in native human neutrophils. Healthy volunteers were genotyped and the adhesion of neutrophils expressing the PSGL-1 isoforms A/A, A/B and B/B were studied under static and physiologic flow conditions. Homozygous B/B neutrophils attached significantly weaker to P-selectin at elevated shear rates than A/A and A/B neutrophils. No difference was found under static conditions and shear stress below 24 dyn/cm2. B/B neutrophils rolled significantly faster than A/A neutrophils at 12 dyn/cm2 shear stress or higher. There was no difference in the adhesive capacity between A/A an A/B neutrophils. These data support the view that the role of the decamers is to extend the ligand binding domain far above the cell surface to support stable leukocyte adhesion and rolling.

Memory Stick

SMALL-SIZED HIGH-DENSITY LIPOPROTEIN PARTICLES ARE ASSOCIATED WITH REDUCED SURVIVAL RATE IN END-STAGE RENAL DISEASE PATIENTS

A. Zeljkovic[1], J. Vekic[1], **Zorana Jelic-Ivanovic**[1], V. Spasojevic-Kalimanovska[1], S. Spasic[1], S. Simic-Ogrizovic[2], V. Dopsaj[1]

[1]Institute of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, [2]Nephrology Clinic, Clinical Center of Serbia, Belgrade, Serbia

Introduction: Dyslipidemia is commonly seen in patients with end-stage renal disease (ESRD). Small-sized high-density lipoprotein (HDL) particles are feature of atherogenic dyslipidemia, but their predictive role for ESRD progression is uncertain. **Objective:** This prospective study investigates the effect of small-sized HDL particles alone and in combination with increased high sensitivity C-reactive protein (hsCRP) levels on survival of ESRD patients treated by haemodialysis (HD).

Methods: We performed 36 months follow-up study in 122 HD patients. HDL size and subclass distribution were determined by gradient gel electrophoresis, while serum hsCRP and lipid parameters were measured by standard laboratory methods. Baseline characteristics of the patients were evaluated for the prediction of mortality.

Results: The carriers of small-sized HDL particles were more prevalent among patients who died (n=24) compared with those who survived (n=98, $P< 0.05$). Serum hsCRP levels were significantly higher in deceased patients ($P< 0.05$). Univariate Cox regressions analysis showed that patients with small-sized HDL particles had 2.8-fold higher risk of adverse disease outcome ($P< 0.05$). In addition, concomitant presence of small-sized HDL particles and increased hsCRP concentration was significantly associated with reduced survival rate (HR=3.907; $P< 0.05$). Observed relationships persisted after adjustment for serum lipid and lipoprotein concentrations.

Conclusions: Both dyslipidemia and inflammation play significant role in the progression of ESRD. Our results indicate that small-sized HDL particles alone and combined with elevated hsCRP concentrations are independent predictors of reduced survival in HD patients.

Memory Stick

UK INPRACTICE STUDY: CONCORDANCE BETWEEN APOLIPOPROTEIN (APO) B LEVELS AND LDL-CHOLESTEROL AMONG PATIENTS ON STATINS & NON-STATIN TREATMENT

I. Idris[1], H. Tate[2], **Azhar Ahmad**[3], T. Mccormack[4]

[1]Sherwood Forest Hospitals Foundation Trust, Mansfield, [2]MSD, Hoddesdon, [3]Medical, MSD, Welwyn Garden City, [4]Whitby Group Practice, Whitby, UK

Background: ApoB is known to be a superior predictor of LDL-C particle number and CVD risk compared with LDL-C. In patients not taking lipid lowering therapy, current evidence has questioned the concordance between LDL-C with ApoB levels. However the concordance in patients receiving lipid lowering therapies is unclear. This analysis examines the LDL-C and ApoB levels among patients receiving statins and non-statin treatment.

Methods: We performed post hoc analysis of the INPRACTICE* data looking at the concordance between LDL-C with Apo B values. This analysis involved 760 high-risk CVD patients from 34 primary care centres initially treated with simvastatin (S) 40mg at baseline subsequently randomized to adding ezetimibe (E) to S 40mg or changed to atorvastatin (A) 40mg or to rosuvastatin (R) 5-10mg for 6 weeks. Graphs and cross-tabulated quintile tables were assessed.

Results: Based on the post-6-weeks treatment values showed that for all treatment groups there was a similar relationship between LDL-C and ApoB; Pearson correlation coefficients were 0.84(E+S), 0.82(A) and 0.83(R). Proportions of patients who fell into the same quintile were 49%(E+S), 46%(A) and 44%(R). Kappa analysis confirmed fair agreement for all treatment groups; 60(E+S), 0.58(A) and 0.55(R). Proportions of patients achieving ApoB< 90mg/dl were 85%(E+S), 72%(A) and 58%(R); P< 0.0001.

Conclusion: There was a similar concordance pattern between LDL-C and ApoB in patients treated with E+S compared to A and R. A higher percentage of patients achieved an ApoB level of < 90mg/dl whilst treated with E+S compared to A and R

*Presented at ESC 2009

Memory Stick

EVALUATION OF LIPID, INFLAMMATORY AND OXIDATIVE STRESS/ANTI-OXIDATIVE DEFENCE MARKERS FOR THE PREDICTION OF CARDIOVASCULAR RISK

Natasa Bogavac-Stanojevic, Z. Jelic-Ivanovic, S. Spasic, V. Spasojevic-Kalimanovska, J. Kotur-Stevuljevic

Institute of Medical Biochemistry, Faculty of Pharmacy, University in Belgrade, Belgrade, Serbia

Objectives: Atherosclerosis is a complex disease caused by a variety of risk factors such as dyslipidemia, inflammation and oxidative stress. However, none of the aforementioned risk factors used as a single biomarker is able to predict coronary artery disease (CAD) with sufficient accuracy. Measurement of multiple biomarkers may be an effective strategy to improve prediction of CAD.

Methods: The effectiveness of apolipoproteins, lipoproteins, inflammatory and oxidative stress/anti-oxidative defence risk parameters supplementary to Framingham scoring data (FRS) was established in 188 CAD patients and 197 controls. The discriminative ability of the models was evaluated using receiver-operating characteristic (ROC) curves with the predictive probabilities from logistic regression models. The jackknife technique was used to detect the outliers.

Results: The model, consisting of FRS, superoxide dismutase (SOD) and superoxide anion had outstanding discriminative ability with the areas under the curves (AUC) value of 0.924. One more model (FRS plus SOD) had an outstanding AUC value (0.906). All other models had excellent discriminative abilities (AUC values between 0.854 and 0.887). Results obtained using the jackknife technique indicate that our original model estimates were stable and were not affected by influential outlier cases within the study sample.

Conclusions: Our results support the feasibility of using oxidative stress/anti-oxidative defence markers in the clinical laboratory. Prospective studies validating the utility of novel markers in combination with FRS are needed to fully determine the most effective model for coronary risk assessment.

FUNDING: This work was supported by a grant from the Ministry of Science, Republic of Serbia.

Memory Stick

INCREASED PLASMA LEVELS OF NON-HDL-BOUND SPHINGOSINE-1-PHOSPHATE (S1P) IN CORONARY ARTERY DISEASE ARE CAUSED BY ALTERATIONS OF S1P UPTAKE BY HDL

Katherine Sattler[1], S. Elbasan[1], P. Keul[1], M. Elter-Schulz[2], C. Bode[3], M. Gräler[3], M. Bröcker-Preuss[4], T. Budde[5], R. Erbel[6], G. Heusch[1], B. Levkau[1]

[1]Institute of Pathophysiology, University Hospital Essen, [2]Institute for Transfusion Medicine, University Duisburg-Essen, Essen, [3]Institute for Immunology, Hannover Medical School, Hannover, [4]Department of Endocrinology, Division of Clinical Chemistry and Laboratory, University Duisburg-Essen, [5]Clinic of Internal Medicine and Cardiology, Alfried Krupp Hospital, [6]Clinic of Cardiology, West German Heart Center, University Duisburg-Essen, Essen, Germany

Objective: High-density lipoproteins (HDL) are the major plasma carriers for S1P in healthy individuals but their S1P content is unknown for patients with coronary artery disease (CAD).

Methods: S1P was determined in plasma and in HDL isolated from patients with myocardial infarction (MI, n=83), stable CAD (sCAD, n=95) and controls (n=85). The S1P-uptake capacity of HDL isolated from CAD patients and controls as well as of modified isolated HDL and of HDL subfractions was assessed *in vitro*.

Results: HDL-bound plasma S1P levels were dependent on the plasma HDL-C levels. HDL-bound plasma S1P was 1.5-fold lower and non-HDL-bound S1P 8-fold higher in MI and sCAD compared to controls. Non-HDL-bound plasma S1P correlated with CAD symptom severity (CCS) and discriminated patients from controls in ROC analyses. The S1P content of isolated HDL was inversely associated with the non-HDL-bound plasma S1P only in controls but not in CAD patients. *In vitro*, HDL from controls but not from CAD patients acquired exogenous S1P proportionally to their initial S1P content. The uptake of S1P by isolated HDL was dramatically reduced by chlorination or oxidation, and was 3.8-fold less for HDL3 than HDL2.

Conclusions: HDL-bound S1P is decreased and non-HDL-bound S1P increased in CAD. Lower plasma HDL-C levels, altered HDL subfractions and CAD-related modifications of HDL such as chlorination and oxidation may affect the S1P uptake by HDL in CAD.

Memory Stick

FOLATE-PEG-SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES LABELED WITH CY5.5 FOR LUNG CANCER IMAGING

Mohammad Islam[1], M.-K. Yoo[1], H.-T. Lim[2], H.-L. Jiang[2], S.-J. Lee[3], I.-K. Park[3], M.-H. Cho[2], C.-S. Cho[1]

[1]Department of Agricultural Biotechnology, [2]College of Veterinary Medicine, Seoul National University, Seoul, [3]Department of Biomedical Sciences, Chonnam National University Medical School, Gwangju, Republic of Korea

Aims: We have developed a functionalized iron oxide nanoprobe for lung cancer imaging. The nanoprobe was fabricated by immobilizing the folate-conjugated poly(ethylene glycol)(Fol-PEG) onto the surface of superparamagnetic iron oxide nanoparticles (SPIONs) and by labeling with near-infrared (NIR) fluorescence dye Cy5.5. Here, PEG was introduced to prevent the aggregation of SPIONs in physiological medium and capturing of these species by the cells of reticuloendothelial system (RES) and to increase circulation time in the body. Folate conjugation was aimed at tumor-specific nanoprobe targeting through the folate-receptor-mediated endocytosis because folate receptor is often elevated in several cancers including lung cancer. Also, Cy5.5 was used as a fluorophore for optical imaging.

Methods: The particle size, morphology, and crystallinity of Fol-PEG-SPIONs were characterized by electrophoretic light scattering spectrophotometer, field emission scanning electron microscopy, and X-ray diffractometer, respectively. In vitro intracellular uptake of the Fol-PEG-SPIONs-Cy5.5 was assessed by FACS. Their toxicity was also assessed in vitro and in vivo. Further, we checked in vivo imaging after their intravenous administration in lung cancer model mice. After systemic injection of Fol-PEG-SPIONs-Cy5.5, bio-distribution profiles in various tissues were quantitatively determined and visualized.

Results: Fol-PEG-SPIONs-Cy5.5 exhibited excellent stability in physiological medium, lower cytoxicity, and enhanced targeted delivery efficiency to cancer cells both in vitro and in vivo. NIR fluorescence imaging demonstrated that tumor sites were better clearly identified upon systemic injection of Fol-PEG-SPIONs-Cy5.5 than PEG-SPIONs-Cy5.5 without folate.

Conclusion: The above results suggest that Fol-PEG-SPIONs-Cy5.5 can be exploited as in vivo optical imaging agent for lung cancer diagnosis.

Memory Stick

NEW LPL AND APOA5 GEN MUTATIONS IDENTIFIED IN SUBJECTS WITH SEVERE HYPERTRIGLYCERIDEMIA

Isabel De Castro Orós[1], Á.L. García Otín[2], M. Pueyo[1], P. Mozas[1], L. Gómez[3], F. Fabiani[4], L. Álvarez-Sala[5], E. Ros[6], D. Zambón[6], J. Dalmau[7], F. Civeira[2], M. Pocovi[8]

[1]RECAVA, Dpto. Bioquímica y Biología Molecular y Celular,, Universidad de Zaragoza. Instituto Aragonés de Ciencias de la Salud (I+CS), [2]Unidad de Lípidos. Laboratorio de Investigación Molecular, Hospital Universitario Miguel Servet. Insituto Aragonés de Ciencias de la Salud (I+CS), Zaragoza, [3]Sección de Gastroenterología, Hepatología y Nutrición, Hospital San Juan de dios, Barcelona, [4]UGC de Bioquímica Clínica, Hospital Virgen de la Macarena, Sevilla, [5]Unidad de Lípidos, Hospital Gregorio Marañon, Madrid, [6]Unitat de Lipids, Servei d'Endocrinologia i Nutrició., Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic and Ciber Fisiopatología de la Obesidad y Nutrición (CEBEROBN), Barcelona, [7]Unidad de Nutrición y Metabolopatías, Hospital Infantil de la Fe, Valencia, [8]CIBERER, Dpto. Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza. Instituto Aragonés de Ciencias de la Salud (I+CS), Zaragoza, Spain

Introduction: Several genes have been associated with severe Hypertriglyceriemia (SHTG) Lipoprotein lipase (LpL) is a critical enzyme in triglyceride (TG) metabolism. Apolipoprotein C-II (Apo C-II) is required by LpL for its activation. Apo-AV is also needed to regulate TG metabolism. Nowadays, more than 100 mutations have been described in *LPL*, *APOC2* and *APOA5* associated with SHTG.

Objective: The aim of our study was to identify the genetic causes of SHTG.

Methods: We selected 33 unrelated with TG levels higher than 900 mg/dl and without secondary causes of SHTG. Complete sequencing of exons and intron-exon boundaries of *LPL*, *APOC2* and *APOA5* was carried out.

Results: We found 5 variants in *LPL* gene not previously described: H80R (c.907>G), Q262X (c.1452C>T), K287_A288del (c.1502_1507delAAGGCC), G300V (c.1542G>T) and R333H (c.1666G>A); and one in *APOA5*, L253P. No mutations in *APOC2* were found. Moreover, 3 patients presented mutations in *LPL* previously described, two G215E homozygous and one I232S homozygous. The Q262X mutation was found in homozygosis, whereas G300V and R333H variants were found in heterozygosis. The individual who presented the K287_A288del mutation was also carrier of a synonymous variant: T388T. Furtheremore, the L253P in *APOA5* was found in homozygosis. Analyzing these mutations in a normolipidemic control group (100 alleles) revealed no carriers of these mutations.

Conclusions: We have identified SHTG causes in 8 out of 33 (24%) subjects: 5 new and 2 recurrent mutations in *LPL* and one mutation in *APOA5*.

Memory Stick

POOR MENTAL HEALTH, HPA AXIS REACTIVITY AND SUBCLINICAL CORONARY CALCIFICATION

Adrie Seldenrijk[1,2], M. Hamer[2], B. Penninx[1,3,4], A. Steptoe[2]

[1]Psychiatry, VU University Medical Center, Amsterdam, The Netherlands, [2]Epidemiology and Public Health, University College London, London, UK, [3]Psychiatry, Leiden University Medical Center, Leiden, [4]Psychiatry, University Medical Center Groningen, Groningen, The Netherlands

Objective: Poor mental health has been associated with coronary heart disease (CHD). One of the hypothesized mechanisms is HPA-axis dysfunction. We examined the associations between poor mental health, cortisol response to laboratory-induced mental stress and subclinical coronary artery calcification (CAC).

Methods: Participants were 527 CHD-free volunteers (mean age = 63.0 ± 5.7 years), drawn from the Whitehall II cohort. CAC was measured using electron beam computed tomography. Mental health was assessed using the Short Form-36 Mental Health subscale at time of the heart scan (current) and additionally five times over preceding 15 years (chronic). Salivary cortisol was measured in response to mental stressors (Stroop, mirror tracing).

Results: Detectable CAC (some: Agatston score 1-399; severe: Agatston ≥ 400) was found in 56.4% of the sample. After adjustment for sociodemographic and conventional risk factors, chronic but not current poor mental health was associated with a higher risk of severe CAC (per SD decrease: OR_{severe} = 1.47, 95%CI = 1.02-2.12; log Agatston = 0.25 ± 0.11, p=.02 within detectable CAC subpopulation). Poor mental health was associated with blunted cortisol response (per SD decrease: $\beta_{current}$ = -.06, p=.01; $\beta_{chronic}$ = -.05, p=.03). We observed a trend for interaction (p=.097) indicating that persons with poor mental health and high cortisol reactivity showed the highest severe CAC prevalence.

Conclusions: In healthy, older participants, chronic but not current poor mental health is associated with CAC. Although participants with poor mental health generally showed blunted cortisol responses, those with cortisol responsiveness were at higher risk for severe CAC.

Memory Stick

ASSOCIATION BETWEEN LP(A) AND HDL CHOLESTEROL ON GENERAL POPULATION

Stamata Pagoni[1], C. Charalampopoulos[1], D. Syrigos[2], E. Zoulias[1], C. Voulgari[2], B. Papalimneou[2], A. Mousouli[1], S. Paximadas[2]

[1]Lipid Unit, General Hospital of Athens G.Gennimatas, [2]Lipid Unit, General Hospital of Athens ELPIS, Athens, Greece

Introduction: Latter between risk factors for cardiovascular diseases included the increase of levels Lp(a) cholesterol. On the contrary, the elevated plasma levels of HDL cholesterol are a negative factor for coronary artery disease and stroke.

Purpose: The aim of this study was to examine the possible role and association of Lp(a) on HDL on general population.

Material and **Methods:** The study included 100 out patients, aged 35-81 years who examined in the Lipid Unit. Investigated the Lp(a) and HDL levels and correlated. All were whites and not taking any medication. All results were analyzed in the same laboratory. Statistical analysis was performed using a SPSS 11,0.

Results: Lp(a): 32,7±30,4 mg/dl (3,01 - 164,7), HDL: 45,6±6 (12 - 143).

Lp(a) < 10 mg/dl: HDL 55,16, p= 0,00

Lp(a) < 15 mg/dl: HDL 47,13, p= 0,08

Lp(a) < 20 mg/dl: HDL 45,92, p= 0,21

Lp(a) < 25 mg/dl: HDL 45,40, p= 0,31

Lp(a) < 30 mg/dl: HDL 45,02, p= 0,23

Lp(a) < 35 mg/dl: HDL 44,58, p= 0,32

Conclusions: The study shows that when increased the Lp(a) levels, then decreased the HDL levels.

Memory Stick

THE CLICAL IMPLICATIONS OF ADIPOCYTOKINE,SERUM RESISTEN IN DIABETICS WITH CORONARY ARTERY DISEASE

Waleed Fatehy, A. Abd El Ghany, A. Abd El Kader, G. Kamal, M. Soliman

Menofyia, Sheben el Kom, Egypt

Background: Diabetics with CAD have an unfavorable outcome even after PCI or CABG.So, investigations of novel atherogenesis markers such as serum resisten may have a potential role in early assessment of cardiovascular risk in such patients.

Aim of the work: To assess serum resisten levels in diabetics with: stable CAD, ACS & healthy control.

Subjects and methods: The present study included fourty patients with diabetes, twenty with CAD,group1(A with stable CAD and B with ACS), Twinty without CAD,group11 and twelve healthy as control, group111. All subjects underwent:history taking and physical examinations ECG,Stress testing and or coronary angiography

Laboratory investigations:glycated HB, creatinine, lipid profile, HsCRP, Resisten.

Results: Diabetics have significantly higher BMI, glycated HB, total cholesterol, TG, LDL, HsCRP & Resisten than healthy control. There was no significant deference between both diabetic groups as regards, BMI, lipogram, but group1 have significantly higher HsCRP & Resisten. Diabetics with stable CAD have significantly higher age,glycated HB and significantly lower HsCRP& Resisten than those with ACS. Resisten was correlated with BMI, glycated HB, cholersterol, LDL, TG, HDL &HsCRP in diabetic patients.

Conclusion: Diabetics with CAD have significantly higher serum resisten than diabetics without CAD & healty control. Also, diabetics with ACS have significantly higher serum resisten than diabetics with stable CAD. Serum resisten may serve as a biomarker of increased atherogenic risk in diabetic patints

Memory Stick

GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A NEW LOCUS FOR CORONARY ARTERY DISEASE ON CHROMOSOME 10P11.23

Jeanette Erdmann

Medizinische Klinik II, Universität Lübeck, Lübeck, Germany

Recent GWAS identified 13 loci for CAD/MI. However, these loci explain only a small proportion of the genetic variability of these pertinent diseases. We sought to identify additional CAD/MI loci by applying a three-stage approach.

We genotyped n=1,157 MI cases and n=1,748 controls from a population-based study population (German MI Family Study (GerMIFS) III (KORA)) with genome-wide SNP arrays. At this first stage n=462 SNPs showed association with MI at $P < 1x10^{-3}$ in two-sided trend tests. In a second stage, 415 of these SNPs were evaluated *in silico* in two independent GWA samples, the GerMIFS I (875 cases/ 1,644 controls) and GerMIFS II (1,222 cases/ 1,298 controls). Nine SNPs, representing three regions, displayed consistent replication in this *in-silico* analysis ($P < 0.05$ for each GWA sample): five SNPs at 9p21.3, a well-known CAD/MI locus, two SNPs at 10p11.21 and two SNPs at 2p24.3. Wet-lab replication, i.e. the third stage, of SNP rs3739998 (representing the novel locus at 10p11.21, p.S1002T in *KIAA1462* gene) in additional 5,790 cases and 5,302 controls confirmed the association ($P = 9.54x10^{-4}$), but not for the 2p24.3 locus. The combined *P*-value across all stages for SNP rs3739998 is $P = 3.52x10^{-11}$ (OR = 1.15 (1.11 - 1.20)).

Analysis of a GWA study followed by *in-silico* and wet-lab replication steps identified the *KIAA1462* gene, encoding a yet uncharacterized protein, on chromosome 10p11.23 with genome-wide significant association for CAD/MI. Further studies are needed to characterize the functional role of this locus in the etiology of these diseases.

Memory Stick

HOMOARGININE INDEPENDENTLY PREDICTS TOTAL AND CARDIOVASCULAR MORTALITY IN INDIVIDUALS WITH ANGIOGRAPHIC CORONARY ARTERY DISEASE

Winfried März[1,2], A. Meinitzer[2], S. Pilz[3], C. Drechsler[4], V. Krane[4], M. Kleber[1], J. Fischer[5], B. Winkelmann[6], B. Böhm[7], E. Ritz[8], C. Wanner[4]

[1]Synlab Medizinisches Versorgungszentrum Heidelberg GmbH, Eppelheim, Germany, [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, [3]Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria, [4]Department of Medicine, Division of Nephrology, University of Würzburg, Würzburg, [5]Mannheim Institute of Public Health, Medical Faculty Mannheim, Mannheim, [6]Division of Endocrinology and Diabetes, Ulm University, Ulm, [7]Frankfurt Cardiology Centre, Frankfurt/Main, [8]Division of Nephrology, Ruperto Carola University Heidelberg, Heidelberg, Germany

Introduction: Homoarginine is an amino acid derivate which increases nitric oxide levels and may prevent cardiovascular damage. We therefore evaluated whether homoarginine levels are associated with cardiovascular risk factors and mortality.

Methods: We measured homoarginine levels in 3305 patients from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study, who had undergone coronary angiography at baseline (1997 to 2000).

Results: Homoarginine levels declined with increasing age and were significantly higher in males compared to females. Impaired renal function, N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) and markers of endothelial dysfunction (ICAM-1 and VCAM-1) were significantly associated with homoarginine deficiency but we found no association with coronary artery disease, arterial hypertension and diabetes mellitus. During a median follow-up time of 7.7 years, 766 patients died including 482 deaths due to cardiovascular causes. Cox proportional hazard ratios (with 95% CI) for all-cause and cardiovascular mortality were 3.03 (2.44-3.77) and 4.13 (3.06-5.57), respectively, when comparing the first with the fourth homoarginine quartile. After adjustments for cardiovascular risk factors and indicators of malnutrition, these hazard ratios remained significant for all-cause mortality with 2.04 (1.56-2.65) and for cardiovascular mortality with 3.00 (2.11-4.28; $p < 0.001$ for all hazard ratios).

Conclusions: Homoarginine deficiency is a significant and independent risk factor for all-cause and cardiovascular mortality in patients referred for coronary angiography.

Memory Stick

IMPORTANCE OF NON-HUMAN PRIMATES IN PRE-CLINICAL EVALUATION OF LXR AGONISTS

Heather McKinnon[1], S. Miller[1], N. Barnett[1], K. McGregor[1], D. Mallinson[1], H. Davidson-Smith[1], J. Bennett[2], H. Davis[3], D. Black[1]

[1]Pharmacology, [2]Chemistry, Merck, Glasgow, UK, [3]Pharmacology, Merck, Kenilworth, NJ, USA

LXR agonists provide an exciting opportunity for the treatment of metabolic diseases such as atherosclerosis. LXR has a central role in lipid metabolism to increase cholesterol efflux from macrophages via upregulation of ABCA1 and to increase circulating HDL, promoting the reverse cholesterol transport pathway. However, clinical development of LXR agonists has been hampered by their potential to adversely effect lipid balance, causing an increase in pro-atherogenic hepatic and plasma lipids. We have demonstrated that the lipid liability of an LXR agonist can be predicted from studies in cell systems. By measuring the expression of lipogenic genes and lipid accumulation, and comparing to the potency of the compound to upregulate ABCA1 mRNA expression, an apparent safety window can be identified.

In order to establish how these in vitro effects translated in vivo, we tested a potent selective LXRab agonist in mice, rabbits and non-human primates (cynomolgus monkeys). Interestingly, when tested in mice and rabbits, the compound appeared to have a safety window between the desired potency on ABCA1 mRNA upregulation and the unwanted lipid side effects on triglyceride and LDL cholesterol. However, unlike this small animal data, in the cynomolgus monkey the compound did not show a safety window between these two effects; instead the in vitro cell data better predicted the in vivo responses. In summary, these data indicate that lipid liability can be assessed in robust cell systems in vitro, but caution should be taken in interpreting data from small laboratory animals.

Memory Stick

IMPACT OF PLAQUE HAEMORRHAGE ON PLAQUE STRESS IN PATIENTS WITH CAROTID ARTERY DISEASE- A PATIENT-SPECIFIC MAGNETIC RESONANCE IMAGING STUDY

Umar Sadat, Z. Teng, V. Young, J. Gillard

University Department of Radiology, University of Cambridge, Cambridge, UK

Introduction: High resolution magnetic resonance (MR) imaging can be used to assess the atherosclerotic plaque component-dependent biomechanical stresses.

Aims: The aim of this study was to assess the effect of plaque haemorrhage (PH) and age of PH on the plaque stresses by performing patient-specific MR-based computational simulations.

Methods: 27 symptomatic patients and 23 asymptomatic patients underwent high resolution MR imaging of their atherosclerotic carotid arteries. Axial T_1, T_2-weighted, STIR and proton density-weighted images covering the entire carotid plaque were acquired. Manual segmentation of plaque components was done. Patients with MR evidence of PH were only included for this study. Critical stress was generated using finite element method and solved in ADINA 8.5 (ADINA, Inc.).

Results: The median critical stress of symptomatic patients with fresh PH was 159kPa (IQR: 114-253). When the simulation was repeated with fresh PH replaced with lipid pool, the stress was 118kPa (IQR: 79 to 189) (p=0.001), showing that fresh PH caused a 26% (IQR: 13-50) increase in the critical stress from baseline. The simulation performed with chronic haemorrhage replacing fresh PH gave a stress of 118kP (IQR: 79-189), (p=0.001). This indicated a 30% (IQR: 19-44) reduction in the critical stress. The critical stress of asymptomatic patients with chronic PH was 145kPa (IQR: 104-188). The simulation performed with chronic PH replaced with lipid pool produced a critical stress of 152kPa (IQR: 109-204). There was no significant difference between these two states (p=0.65).

Conclusions: Age of the plaque haemorrhage significantly affects biomechanical stresses within atherosclerotic plaques.

Memory Stick

BENEFICIAL EFFECT OF FLAXEED OIL SUPPLEMENTATION IN SUBJECTS WITH METABOLIC SYNDROME

Diana Tint[1], M. Anghel[1], L.M. Fischer[2], M.D. Niculescu[3]

[1]University of Transilvania, School of Medicine, Brasov, Romania, [2]University of North Carolina at Chapel Hill, Nutrition research Institute at Kannapolis, [3]University of North Carolina at Chapel Hill, Department of Nutrition, Chapel Hill, Kannapolis, NC, USA

Background: Flaxeed oil is a rich source of a-linolenic acid (ALA), and may have some beneficial effects on ameliorating cardiovascular risk factors, but the effect on metabolic syndrome (MS) is unclear.

Objective: To assess the effect of Flaxeed oil supplementation in subjects with MS.

Method: Prospective, double-blind, placebo-controlled, randomized trial in male subjects (sbj) with MS divided in two groups: 10 sbj (ALA group) received 2400 mg flaxseed oil/day and 10 sbj (CT group) received 2576 mg corn oil (placebo)/day for 3 months.

Clinical parameters and biochemical investigations (glucose, insulin, HOMA-R index, lipoproteins, oxLDL and hsCRP) were assessed at days 0 and 90. Data analysis was performed using bivariate analysis (linear fit between days 0 and 90) and logistic regression.

Results: The BMI increased at day 90 vs day 0 in CT group while it remained constant in ALA group (1.1 vs -0.3, $p< 0.05$). The level of total cholesterol (mg/dl) at day 90 was higher than at day 0 in CT with significant correlation (209 vs 203.6, $p< 0.01$). The insulin level and the HOMA-R index decreased in ALA group with significant correlation on day 90 vs day 0 (14.55 vs 12.92, $p< 0.05$ and 5.29 vs 4.23 $p< 0.0242$), while no differences in glucose level were found. There were no changes in other biochemical parameters.

Conclusion: The administration of low doses of Flaxseed oil improved insulin sensitivity and decreased BMI, suggesting a protective effect against the natural evolution of MS regarding insulin sensitivity and body weight increase.

Memory Stick

SERUM CONCENTRATIONS OF HSP60 AND HSP70 IN PATIENTS WITH CEREBRAL ATHEROSCLEROSIS

Ruzica Galovic[1], Z. Flegar-Mestric[2], S. Perkov[2], V. Vidjak[3], K. Barisic[1]

[1]University of Zagreb Faculty of Pharmacy and Biochemistry, [2]University Hospital Merkur, Dept. of Clinical Chemistry, [3]University Hospital Merkur, Institute for Radiology, Zagreb, Croatia

Previous studies suggest that heat shock proteins (Hsp) have a contributory role in atherosclerosis development. The intention of this study is to investigate serum concentrations of Hsp60 and Hsp70 in patients with cerebral atherosclerosis.

A group of patients with cerebral atherosclerosis, evaluated by digital subtraction angiography, with >50% stenosis of cerebral artery (n=50) was compared to the control group (n=25) concerning serum concentrations of Hsp60 and Hsp70. Hsp60 and Hsp70 were assayed by commercially available ELISA kits (Stressgen, USA).

Serum Hsp60 was detectable in 74% of subjects with concentrations ranging from 3.13 µg/l to 391.34 µg/l (mean, 33.96; median, 14.25 µg/l). There was no significant difference in Hsp60 levels in patients compared to controls. The high Hsp60 level group (above the median value) contained 61% cerebral stenosis patients, whereas the low Hsp60 group had 68% cerebral stenosis patients, without significant difference in proportions.

Serum Hsp70 was detectable in 52% of subjects with concentrations ranging from 0.09 µg/l to 5.47 µg/l (mean, 0.424; median, 0.024 µg/l). There was no significant difference in Hsp70 levels in patients compared to controls. The high Hsp70 level group (above the median value) contained 55% cerebral stenosis patients, whereas the low Hsp70 group had 79% cerebral stenosis patients ($p < 0.05$).

There was no difference in serum Hsp60 and Hsp70 concentrations in the group of patients with cerebral atherosclerosis compared to the control group. Patients with cerebral atherosclerosis had greater prevalence of low Hsp70 serum levels. The deficiency in serum Hsp70 concentration could influence cerebral atherosclerosis development.

Memory Stick

HYPERTRIGLYCERIDEMIC WAIST: AN ALTERNATIVE TO THE METABOLIC SYNDROME? RESULTS OF THE IMAP STUDY (MULTIDISCIPLINARY INTERVENTION IN PRIMARY CARE)

R. Gomez-Huelgas[1], **Rosa Bernal-Lopez**[2], A. Villalobos[1], J. Mancera-Romero[3], A. Baca-Osorio[3], S. Jansen[1], R. Guijarro-Merino[1], F. Salgado[1], F.J. Tinahones[4], M. Serrano-Rios[5]

[1]Hospital Regional Universitario Carlos Haya, [2]Hospital Clinico Universitario Virgen de la Victoria (Fundacion IMABIS). CIBEROBN, [3]Health Center 'Ciudad Jardin', [4]Hospital clinico Universitario Virgen de la Victoria (Fundacion IMABIS). CIBEROBN, Málaga, [5]Hospital Clínico Universitario San Carlos, Madrid, Spain

Aim: To study the prevalence of hypertriglyceridemic waist (HTGW) in an adult Spanish population and its association with type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).

Methods: We undertook a cross-sectional analysis in a random sample of 2270 individuals (18-80 years of age). HTGW was diagnosed using anthropometric criteria for the European population and fasting plasma triglycerides ≥1.71 mmol/L.

Results: The prevalence of HTGW was 14.5% (men: 18.2%, women: 10.8%).HTGW was associated with older people, a low educational level and, in men, with a sedentary lifestyle ($p< 0.001$). Subjects with HTGW had higher levels of total cholesterol, LDL-c and uric acid, lower levels of HDL-c, a higher blood pressure, a greater degree of obesity, and a higher prevalence of T2DM (20.00% vs. 6.4%, $p< 0.001$) (OR 3.61; 95% CI, 2.60-5.01) and CVD (8.5% vs. 3.4%, $p< 0.001$) (OR 2.63; 95% CI, 1.66-4.16). The degree of concordance between HTGW and the metabolic syndrome (MS) was moderate, with both the ATP-III and the IDF criteria (kappa 0.51 and 0.58, respectively). The subjects with isolated HTGW as compared to the subjects with isolated MS (ATP-III) were younger, had greater levels of total cholesterol, LDL-c and triglycerides and a lower prevalence of obesity, high blood pressure and dysglycemia.

Conclusion: HTGW is a phenotype of cardiometabolic risk prevalent in the adult population in our environment. HTGW may be an alternative to the MS to detect the population at risk for T2DM and CVD, especially in young persons who have no criteria for the MS.

Memory Stick

INTERLEUKIN 15 (IL15) DEFICIENCY DECREASES ATHEROSCLEROSIS IN APOLIPOPROTEIN E KNOCKOUT MICE (APOE KO)

Omid Dadoo[1,2], A. Ashkar[1], C. Richards[1], B. Trigatti[1,2]

[1]McMaster University, [2]Thrombosis and Atherosclerosis Research Institute, Hamilton, ON, Canada

Objectives: To test the role of interleukin 15, a key NK/ NKT cell survival factor on atherogenesis.

Methods: *il-15*$^{-/-}$, *il-15*$^{+/-}$ and *il-15*$^{+/+}$ mice were generated on *apoE*$^{-/-}$ background and fed a normal chow diet until 25 weeks of age. Lipoprotein cholesterol profiles were determined by cholesterol assay. Leukocyte populations were analyzed by flow cytometry, and cytokines were measured by ELISA. Cellular and molecular components of atherosclerotic plaques were detected by immunostaining.

Results: IL-15 deficiency resulted in a reduction in atherosclerosis in both the aortic sinus and descending aorta, despite increased plasma lipoprotein cholesterol. *il-15*$^{+/-}$ mice exhibited intermediate levels of atherosclerosis in the aortic sinus. IL-15 deficiency resulted in a lack of NK cells and a reduction in monocyte, and CD8α^{+} T cell populations. Levels of CD11b^{+} monocytes, and CD8α^{+} T cells were reduced in atherosclerotic lesions from *il-15*$^{-/-}$ mice compared to those from *il-15*$^{+/+}$ mice. Treatment of macrophages with IL-15 in vitro induced activity of iNOS, and expression of IL-6, MCP-1, and RANTES. Plaques from *il-15*$^{-/-}$ mice exhibited reduced MCP-1 than those from *il-15*$^{+/+}$ mice. Furthermore in vivo IL-15 deficiency caused a significant reduction of pro-inflammatory SAA levels. Finally we generated *il-15 transgenic* mice on *apoE*$^{-/-}$ background to evaluate the effect of IL-15 over-expression in vivo on atherogenesis.

Conclusion: IL-15 plays a significant role in promoting atherosclerosis development. This may involve its effects as a survival factor of NK/NK-T cells, pro-inflammatory T cell maturation/recruitment, modulation of monocyte levels, and/or direct activation of macrophages.

Memory Stick

PLATELET FUNCTIONAL ACTIVITY AND INFLAMMATION DURING CHD

Liudmila Buryachkovskaya, A. Sumarokov, I. Uchitel, T. Shirokova, E. Gupalo

Russian Cardiology Research Complex, Moscow, Russia

Introduction: Platelets integrate inflammation, thrombosis and atherogenesis. Inflammation induces platelet activation, their interaction with leucocytes, erythrocytes and endothelial cells. Different platelet subpopulations take part in chronic inflammation in the vessel wall.

Methods: There were included 84 patients (pts) with coronary heart disease

(CHD) and 18 healthy volunteers (HV) as a control group. Their platelet morphology, number of circulating leukocyte-platelet (LPA) and erythrocyte-platelet (EPA) aggregates was estimated by scanning electron microscopy.

Spontaneous (SPA) and ADP-induced (0.1-5.0μM) platelet aggregation (IPA) were measured by laser aggregation analyzer “Biola”, Russia. The level of hs-CRP was assessed by nephelometry.

Results: In whole blood of CHD pts there was: high IPA; discoid platelets decrease ($p< 0.01$) and activated platelets increase ($p< 0.05$) as compared with HV; increase of mean platelet volume (12.6±2.7fl vs 8.4±0.7fl in HV). There was observed also the presence of big reticulated platelets (BRP) (1.8±1.6% vs 0% in HV) which possessed high haemostatic potential. BRT were found in EPA but not in LPA. Their number in 33% CHD pts was higher then 2% and correlated with elevated SPA and hs-CRP level (1.1±0.3 ng/l in pts with number of BRP more than 2% vs 0.3±0.2 ng/l in pts with number of BRP less than 2%). The elevated CRP level also was attended by LPA increase. The presence of BRP decreased the pts sensitivity to aspirin.

Conclusion: The BRP appearance in blood is connected with changes in megakaryocytopoiesis as a result of inflammation. EPA and LTA have an influence on platelets sensitivity to antiaggregants.

Memory Stick

SMOKING IS THE MAIN DETERMINANT OF SLOW CORONARY FLOW IN PATIENTS WITH NORMAL CORONARY ARTERIES

Yaron Arbel, E. Rind, A. Halkin, S. Berliner, I. Herz, G. Keren, S. Banai, A. Finkelstein

Tel Aviv Medical Center, Tel Aviv, Israel

Introduction: Reduced myocardial perfusion assessed during angiography has been shown to be a negative prognostic sign in patients with myocardial infarction. It has been attributed to various reasons such as: distal embolization, hyperviscocity, endothelial dysfunction, coronary spasm or inflammation. In order to evaluate the factors associated with slow flow, we recruited patients that had normal coronaries per angiography in order to exclude distal embolization as a cause of coronary slow flow. In this study, we evaluated the factors affecting coronary blood flow

Methods: . We recruited 114 consecutive patients who underwent angiography and were found to have normal coronaries per angiography. Each patient's angiogram was evaluated by 2 blinded specialists who graded the patients' TIMI frame count, TIMI flow grade and Clearance scores.

Results: Corrected TIMI Frame Count which evaluates epicardial blood flow and Clearance Rate Score which evaluates the microcirculation blood flow were highly correlated (r=0.6, p=0.0001). Their range was 14 fold (10-143 frames) with 30% of the patients with slow coronary blood flow. These correlations persisted in all the different coronary arteries supporting the assumption that slow flow is a systemic problem and not a local problem due to spasm of a specific artery. No correlation was found between coronary blood flow and different biomarkers of inflammation (CRP, WBC), rheology (fibrinogen), endothelial dysfunction (ICAM, VCAM, E-selectin), or metabolic parameters (HDL, LDL, triglycerides, HbA1c). Current smoking was the only determinant correlated with slow flow (r=0.24, p=0.007).

Memory Stick

CARDIOVASCULAR AND RENAL SAFETY OF FENOFIBRATE IN THE FIELD STUDY

Ru-Dee Ting[1], T.M.E. Davis[2], P.L. Drury[3], M. Donoghoe[1], K. Rajamani[1], J.D. Best[4], Y.A. Kesaniemi[5], A.C. Keech[1]

[1]NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, [2]School of Medicine and Pharmacology, University of Western Australia, Fremantle, WA, Australia, [3]Greenlane Clinical Centre, Auckland Diabetes Centre, Auckland, New Zealand, [4]Department of Medicine, Univerity of Melbourne, Melbourne, VIC, Australia, [5]Department of Internal Medicine and Biocenter Oulu, Oulu University Hospital, Oulu, Finland

Background: Fenofibrate, a common lipid-modifying drug, raises creatinine acutely and long-term. We explored whether this has adverse cardiovascular or renal consequences in patients with normal or impaired renal function.

Methods: In FIELD, 9795 patients, 50-75 years with type 2 diabetes, were randomly assigned fenofibrate 200mg daily or placebo for 5 years, after six weeks fenofibrate run-in. The pre-specified outcome for subgroup analysis was total cardiovascular events. Renal function (MDRD) was measured at baseline, close-out and in a washout cohort (n=661) 8 weeks after study close. Additionally, pre-specified end stage renal events were recorded. Analysis was by intention-to-treat.

Results: Overall, fenofibrate reduced total cardiovascular events by 11% (p=0.035), with comparable benefits seen in all eGFR subgroups (p-interaction=0.2), and no safety differences. The greatest absolute risk reduction (7.5%) was observed with baseline eGFR 30-59 ml/min/1.73m^2 (HR 0.68, p=0.035). This group also had the greatest risk reduction for cardiovascular mortality (HR 0.51, p=0.028, p-interaction < 0.03). Treatment reductions on cardiovascular disease were also no smaller among those with the greatest creatinine rise. From baseline to washout, eGFR fell less in those on fenofibrate despite an early sustained rise in creatinine of ~10µmol/L: mean fall 6.9 (p< 0.0001) vs 1.9ml/min/1.73m^2 (p=0.065) sparing 5.0ml/min/1.73m^2 (p=0.0003) over 5 years. End-stage renal disease events were no more common with fenofibrate.

Conclusions: Fenofibrate slows eGFR loss, does not cause renal injury in diabetes nor reduce cardiovascular benefits when creatinine rises. There were large cardiovascular benefits and no renal issues when used in those with moderate renal impairment.

Memory Stick

TO ASSESS THE ATTITUDE AND NEGLIGENCE OF HYPERTENSIVE PATIENTS TOWARDS LIFESTYLE MODIFICATION

Sidra Jawed Zafar[1], S. Tai[1], S. Bashir[1], M.A. Saleem[1], A. Zaki[1], M. Kazmi[1], M. Majeed[1], A. Qasim[2]

[1]Dow Medical College, [2]Cardiology, Civil Hospital Karachi, Karachi, Pakistan

Introduction: Hypertension is one of the most common human diseases prevalent worldwide. Mortality and morbidity due to hypertension has declined in the developed world but in developing countries like Pakistan, the problem is still grave. Life-style modification complementary to medical treatment has been proved to be the most effective means of controlling hypertension and its progression. Data regarding lifestyle modification in our part of the world is deficient.

Objective: To explore the attitude and assess negligence of hypertensive patients towards lifestyle modification.

Methodology: Interview based cross sectional study carried out at in-patient and out patient departments of Civil Hospital Karachi. Sample size is set at 300.

Results: Regarding 300 patients interviewed: mean age of the population was 55.65±13.128 years; with 143 males and 157 females having a mean blood pressure (BP) of 136/88. 53% of the population was uneducated. Majority(31%) checked their BP when symptomatic while only 28% measured it monthly. 78% were taking anti-hypertensive medication regularly. 80.7% of total started dietary modification out of which 79.7% were counseled by doctors. 36% started exercising out of the 46% counseled. 64% reduced their salt intake and 7% stopped it completely. 7.3% of the patients did not start any form of lifestyle modification. Majority of the patients who did not start dietary modification developed complications compared to only 30 patients who had started the modifications.

Conclusion: More awareness should be created among hypertensive patients regarding regular monitoring of BP and adapting positive lifestyle modifications for control of hypertension and its complications.

Memory Stick

FENOFIBRATE REDUCES PERIPHERAL NEUROPATHY IN TYPE 2 DIABETES: THE FENOFIBRATE INTERVENTION AND EVENT LOWERING IN DIABETES (FIELD) STUDY

Kushwin Rajamani[1], M. Donoghoe[1], L. Li[1], R.-D. Ting[1], P. Colman[2], R. Scott[3], M. Laakso[4], A. Keech[1], FIELD study investigators

[1]NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, [2]Melbourne Hospital, Melbourne, VIC, Australia, [3]Christchurch Hospital, Christchurch, New Zealand, [4]University of Kuopio, Kuopio, Finland

Introduction: Peripheral neuropathy, a microvascular complication of diabetes, is a major contributor to lower limb ulcer formation and amputation and their devastating consequences. The aim of this analysis was to evaluate the effect of the lipid-modifying drug, fenofibrate, on neuropathy.

Methods: 9795 patients with type 2 diabetes mellitus were randomised to receive fenofibrate 200 mg/day or matching placebo for 5 years. The presence of neuropathy symptoms was recorded at baseline and sensation tested using a standard monofilament technique, repeated at 2 years and study close. All nontraumatic lower limb amputations were recorded, and classified as major or minor.

Results: 5.8% of participants (564 of 9795) had documented monofilament neuropathy at baseline. By study close, neuropathy had increased to 8.0% of placebo patients, compared with 6.6% of those receiving fenofibrate (between-group difference, adjusted for baseline, *P*=0.02).This reflected an 18% reduction in new neuropathy (5.3% vs 4.4%; OR 0.82, 95% CI 0.67-1.01, *P*=0.06,) and a 40% greater reversal of baseline neuropathy with treatment (42% vs 55%; OR 0.60, 95% CI 0.42-0.88, *P*=0.009). Neuropathy was one of the strongest predictors of amputation, increasing the risk of a first amputation approximately threefold (HR 2.7, 95% CI 1.8-4.1; *P*< 0.001) and a minor amputation even more.

Conclusions: Long-term use of fenofibrate reduced overall neuropathy and increased the reversal of pre-existing neuropathy in type 2 diabetes, although the mechanisms remain unknown. These benefits are likely to partly explain the large 37% reduction seen in the risk of total amputations with fenofibrate in the FIELD study.

Memory Stick

INTRODUCING THE VLDL PERFORMANCE DIAGNOSTIC

Daniël B. van Schalkwijk[1,2,3], B. van Ommen[1], A.A. Freidig[4], J. van der Greef[1,2], A.A. de Graaf[1]

[1]BU Biosciences, TNO Quality of Life, Zeist, [2]Analytical BioSciences, LACDR/Leiden University, Leiden, [3]Netherlands Bioinformatics Institute (NBIC), Nijmegen, [4]Amsterdam Molecular Therapeutics (AMT), Amsterdam, The Netherlands

Lipoprotein diagnostics, such as LDL cholesterol and HDL cholesterol, play an important part in cardiovascular disease prevention. However, detailed lipoprotein profile measurements have been little applied in the clinic, mainly because the relation between each lipoprotein subclass and cardiovascular outcomes is hard to establish. Here, we introduce the 'VLDL performance' diagnostic marker that indicates the status of VLDL metabolism based on computational modeling analysis of lipoprotein data. We derived this status from lipoprotein concentration and metabolic flux data using the previously published Particle Profiler model (1). The VLDL performance diagnostic is defined as the average of two ratios of metabolic processes: the ratio between VLDL direct uptake by the liver and VLDL production, and the ratio between VLDL lipolysis by extrahepatic tissue and VLDL production. We then derived the VLDL performance diagnostic from subjects in which lipoprotein concentrations and metabolic fluxes had been measured. These included 44 normolipidemic subjects, and 44 dyslipidemic subjects with various types of dyslipidemia associated with: kidney disease, hypothyroidism, mixed hyperlipidemia, HIV treatment and small LDL particles. We showed that 'VLDL performance' better indicates differences between normolipidemic and dyslipidemic subjects than LDL cholesterol or triglycerides. This study indicates that 'VLDL performance' is a promising diagnostic marker for indicating various types of dyslipidemia. We will go on to apply our methodology to lipoprotein profiles in large population studies, which should validate its clinical value.

1. van Schalkwijk DB, et al. J Lipid Res 2009;50:2398-2411.

Memory Stick

EFFECTS OF LEWIS LUNG CARCINOMA (LLC)-BEARING ON SERUM AND LIVER LIPID LEVELS AND SERUM LIPID-RELATED CYTOKINE CONCENTRATIONS IN MICE

Masashi Kawasaki

Department of Science of Living, Morioka Junior College, Iwate Prefectural University, Takizawa, Japan

To investigate the effects of Lewis lung carcinoma (LLC)-bearing on lipid metabolism, changes in lipid levels in serum and liver and changes in lipid-related cytokine concentrations in serum were examined in LLC-implanted mice. Mice were implanted with 5×10^6 LLC cells and maintained for 14 days. There were no differences between the normal and tumor-bearing groups in terms of food intake and liver weight. LLC implantation significantly reduced body weight gain. Solid tumors were observed 5-7 days after LLC implantation, and the absolute and relative weights of the solid tumors were 2.78 ± 0.54 g and 13.6 ± 2.9 % of body weight, respectively. Serum total cholesterol and very-low-density lipoprotein plus low-density lipoprotein cholesterol concentrations were seen to be significantly increased, while high-density lipoprotein cholesterol concentration was not significantly different following tumor implantation. Thus, the atherogenic index of the tumor-bearing mice was significantly higher than that of the normal mice. Serum free cholesterol concentration was significantly increased by the tumor implantation, while the esterified cholesterol concentration was not significantly different between the normal and tumor-bearing mice. Thus, the cholesterol ratio in the tumor-bearing group was significantly lower than that in the normal group. Hepatic cholesterol content was not significantly different between the normal and tumor-bearing mice. The LLC implantation significantly decreased the hepatic triglyceride content. Serum tumor necrosis factor-α and interleukin-10 concentrations were not affected by the LLC implantation. These results suggest that in LLC-bearing mice, some lipid levels in the serum and liver undergo change, resulting in abnormal lipid metabolism.

Memory Stick

TOTAL CHOLESTEROL, LIPOPROTEINS, AND APOLIPOPROTEINS AS PREDICTORS OF CORONARY HEART DISEASE; 23-YEARS FOLLOW-UP OF THE EDINBURGH HEART DISEASE PREVENTION STUDY

Jennifer Bolton, J. Price, N. Anderson

Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK

There is debate as to whether the ratio of high density lipoprotein(HDL)/total cholesterol or apolipoprotein (Apo)AI/ApoB has better discrimination in coronary heart disease (CHD) risk prediction. The Edinburgh Heart Disease Prevention Study is a population-based, prospective cohort of men aged 30-59 with 23 years follow-up, and baseline measures of total cholesterol, triglycerides, high and low density lipoprotein, and apolipoproteins AI, AII and B. The aim of this analysis was to compare various lipids and lipid ratios in prediction of CHD events, to determine which best improved discrimination of a CHD risk model. When investigating age-adjusted lipid fractions individually, ApoB had the greatest standardised odds ratio (OR) for severe CHD (1.73). Ratios of HDL/total cholesterol and ApoAI/ApoB had similar age-adjusted standardised OR for severe CHD (2.04 and 2.17, respectively) and the ratio of ApoAI/total cholesterol had the greatest standardised OR (2.33). Severe CHD was defined as fatal or non-fatal myocardial infarction, or coronary bypass, revascularization or angioplasty. This finding remained for any form of CHD, although the OR were greater for severe CHD. Using Cox proportional hazards to develop receiver operating characteristic curves found that there was little difference in the discrimination, measured by area under the curve (AUC), when using HDL/total cholesterol or ApoAI/total cholesterol (0.750 and 0.766, respectively), but both of these were superior to ApoAI/ApoB (0.630) for severe CHD. The corresponding AUC showed little difference (0.687, 0.692, 0.684) for any CHD. In this population, ApoAI/total cholesterol and HDL/total cholesterol were better predictors of severe CHD than ApoAI/ApoB.

Memory Stick

PARAOXONASE-1 ACTIVITY IN PATIENTS WITH ANGIOGRAPHICALLY ASSESSED PERIPHERAL ARTERIAL DISEASE

S. Perkov[1], M.M. Kardum Paro[1], V. Vidjak[2], K. Novačić[2], **Zlata Flegar-Meštrić**[1]

[1]Institute of Clinical Chemistry, [2]Institute for Radiology, Merkur University Hospital, Zagreb, Croatia

Background: Low serum paraoxonase 1 (PON1) activity is a biochemical risk factor for atherosclerotic disease.

Aim: of the study is to compare the serum PON1 activity and HDL cholesterol (HDL-C) concentration in patients with angiographically assessed obliteration or peripheral arterial stenosis of different degrees and matched control subjects.

Patients and methods: The venous blood samples were collected by venipuncture from 133 healthy individuals (median age 58 years, range 43-82) and 203 patients with symptomatic peripheral artery disease (median age 64 years, range 40-88), with peripheral arteries obliteration (N=164) or >50% of peripheral arteries stenosis (N=39), assessed angiographically. Paraoxon was used as a substrate for measuring basal or sodium chloride (NaCl)-stimulated PON1 activity. For HDL-C concentration determination direct method based on selective inhibition of the non-HDL fraction was used. The Man-Whitney U test was applied to evaluate the difference between the groups ($p < 0.05$ was considered statistically significant).

Results: Compared to control subjects basal (median values 87 vs. 130 U/L; range 17-495 vs. 46-670 U/L) and stimulated (median values 170 vs. 248 U/L; range 36-984 vs. 90-1184 U/L) PON1 activity and HDL-C concentration (median value 1.1 vs. 1.6 mmol/L; range 0.6-2.4 vs. 0.8.-3.4 mmol/L) were significantly lower in patients with angiography assessed stenosis >50% or obliterations. There were no statistically significant difference between these two group of patients.

Conclusion: The obtained results suggest that lower serum basal and stimulated PON1 activity, as well as HDL -C concentration could be used as biochemical risk factors for peripheral arterial disease.

Memory Stick

EVALUATION OF THE EFFECT OF NICOTINIC ACID (NIACIN) ON ELEVATED LIPOPROTEIN(A) LEVELS (NICOLA STUDY)

Anja Vogt[1], U. Kassner[2], C. Jonatat[2], E. Giannakidou-Jordan[2], J. Müller-Nordhorn[3], S. Willich[4], M. Müller-Riemenschneider[4], C. Kolbe[2], E. Steinhagen-Thiessen[2]

[1]Internal Medicine, LMU Munich, Munich, [2]Metabolic Care Center, [3]Berlin School of Public Health, [4]Institute for Social Medicine, Epidemiology, and Health Economics, Charite University Medicine Berlin, Berlin, Germany

Introduction: Lipoprotein(a) (Lp(a)) is associated with an increased risk of cardiovascular diseases and until now niacin is the sole established pharmacological option to lower Lp(a). Niacin has effectively reduced Lp(a) as a secondary endpoint in studies including patients with primary hyperlipidemia. However, there have been no studies designed to primarily investigate the effect of niacin on levels of Lp(a) above 30 mg/dl. It is also unknown whether baseline Lp(a) level and/or Lp(a) phenotype as well as concurrent therapy with statins are associated with the response to niacin.

Aim of the study: The primary objective is to evaluate the effectiveness of niacin in lowering Lp(a) in patients with levels > 30 mg/dl in comparison to placebo. Secondary objectives besides safety, tolerability, and medication adherence are: (a) mean change in plasma lipids levels, (b) mean change in blood glucose levels, (c) health-related quality of life, and (d) cumulative disease-related costs.

Methods: Randomised (2:1), multicentre, placebo-controlled, 2-arm, parallel group, phase III, intervention study. Subjects with Lp(a) levels > 30 mg/dl will receive niacin (beginning with 500 mg up to 2000 mg per day) or placebo over 20 weeks.

Results: The study is now closed; 122 patients were included. No major adverse events occurred and results are pending.

Perspective: In case of an effective reduction of elevated levels of Lp(a) by niacin, further studies are needed to determine the long-term effectiveness of niacin in reducing incidence and progression of cardiovascular disease in patients with elevated levels of Lp(a).

Memory Stick

UNMASKING CHRONIC MUSCLE DISEASE IN 3 PATIENTS WITH STATIN INDUCED MYOPATHY

Eleni Giannakidou-Jordan[1], A. Rosada[2], U. Kassner[3], H. Knoblauch[4], S. Spuler[4], E. Steinhagen-Thiessen[5]

[1]SWC, Lipidambulanz / Lipidapherese, Charite University Medicine Berlin, berlin, [2]SWC, Lipidambulanz / Lipidapherese, Charité Universitätsmedizin Berlin, [3]SWC, Lipidambulanz / Lipidapherese, Charite Berlin, Campus Virchow Klinikum, [4]Muscle Research Unit, Experimental and Clinical Research Center, [5]SWC, Lipidambulanz / Lipidapherese, Charite University Medicine Berlin, Berlin, Germany

Background: Statins are ingested by millions of people to reduce cardiovascular risk, and about 5% of them develop skeletal muscle symptoms and elevations in creatine kinase (CK) . A variant in the gene SLCO1B1 increases the susceptibility for this side effect 5-fold. We reasoned that variants in other genes could occur in patients with CK elevations and muscle complaints.

Case reports: We saw three patitents with no history of skeletal muscle disorder before initiation of statin treatment. All of them developed physical activity induced myalgia and CK elevation on stain therapy. None of them had a positive family history for muscular disease.

Muscle biopsy was done in two of them. The findings were consistent with myotonic dystrophy type-2. We genotyped all 3 patients for the ZNF9 gene. Each patient had the typical molecular defect underlying myotonic dystrophy type-2.

Discussion: Nothing indicated that our 3 patients had an underlying chronic muscle disease until we began statin therapy. Muscular Symptoms persisted after cessation of statin therapy. Possibly statins unmasked myotonic dystrophy type-2 in our patients. This is an autosomal dominant disorder characterized by proximal muscle weakness, myotonia, and myalgias. The exact mechanisms of somatic instability unterlying this disease nor the time required for altered muscle histology to develop are not known, but we note that one possible explanation for our results is that statins caused CCTG expansion.

Conclusion: Our results argue for measuring CK levels before prescribing statins.

Memory Stick

EVIDENCE FOR AN INSULIN-SENSITIVE, LDLR-INDEPENDENT MECHANISM OF PLASMA LIPID CLEARANCE IN HUMANS

Alexander Laatsch[1], A. Treszl[2], J.A. Cooper[3], R.J.F. Loos[4], N.J. Wareham[4], V. Nicaud[5], I.N. Day[6], T. Gaunt[6], M. Kumari[7], K. Wegscheider[2], P.J. Talmud[3], on behalf of the EARSII, BWHHS, and WHII Study Groups

[1]Department of Biochemistry and Molecular Biology II, [2]Department of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Division of Cardiovascular Genetics, University College School, London, [4]MRC Epidemiology Unit, Addenbrooke's Hospital, Cambridge, UK, [5]INSERM, U525, Université Pierre et Marie Curie-Paris 6, Paris, France, [6]MRC Centre (CAiTE) and Bristol Genetic Epidemiology Laboratories, University of Bristol, Bristol, [7]Department of Epidemiology, University College School, London, UK

Objective: Postprandial lipids are rapidly cleared by the liver, mediated by the low-density-lipoprotein-receptor (LDLR). Recently the LDLR-related protein 1 (LRP1) was identified as playing a major role in the efficient postprandial clearance after stimulation by insulin. LRP1 internalises lipoproteins via apolipoprotein (apo) E as does the LDLR, which additionally binds apoB100. To dissect the contributions of LRP1 and LDLR, studies of individuals of known apoE genotype were analysed. Since apoE2 binds poorly to the LDLR, apoE2 carriers should identify LDLR-independent internalisation.

Methods: Triglyceride levels in *APOEε2* carriers and non-carriers from three prospective UK studies (n=7235) were compared in meta-analysis after stratification by HOMA-IR, a surrogate marker for insulin sensitivity. Additionally, the young, healthy collective of EARSII (n=690) was divided into those with high and low glucose sensitivity, after an oral glucose tolerance test. The lipid response after an oral fat tolerance test was then compared depending on *ε2* carriage.

Results: In meta-analysis, TG levels in the highest tertile of HOMA-IR were higher in *ε2* carriers than in non-carriers (p=0.01). In the EARSII group with lesser insulin sensitivity, *ε2* carriers showed reduced TG clearance (p=0.04).

Conclusions: Our results identify an insulin-dependent mechanism of lipid clearance. Based on our previous results we propose this is mediated by LRP1, in parallel to basal LDLR uptake. Both processes are able to compensate for each other in a situation of insulin-resistance or *ε2* carriage. This compensation may not be present in patients with type III hyperlipoproteinaemia with impaired insulin sensitivity, leading to prolonged postprandial lipeamia.

Memory Stick

EFFICACY OF LIPID APHERESIS IN PATIENTS WITH ELEVATED ISOLATED LIPOPROTEIN(A) CONCENTRATIONS: A RETROSPECTIVE STUDY

Adrian Rosada[1], U. Kassner[1], A. Vogt[2], E. Giannakidou-Jordan[1], H. Berthold[1], E. Steinhagen-Thiessen[1]

[1]Arbeitsbereich Lipidstoffwechsel Lipidambulanz, Charité University Medicine Berlin, Berlin, [2]Ambulanz für Stoffwechselkrankheiten, Lipidapherese, Ludwig-Maximilians-Universität München, Munich, Germany

Introduction: Elevated lipoprotein(a) (Lp(a)) is an independent risk factor for atherosclerosis and cardiovascular events. Regularly performed lipid apheresis has proven to reduce Lp(a) concentrations. Despite some data from observational studies and several case reports, there is a lack of reliable data on the clinical efficacy of lipid apheresis in patients with isolated elevated Lp(a).

Objective: The aim of this study was to investigate whether lipid apheresis administered to patients with elevated concentrations of Lp(a) and severe cardiovascular disease is able to reduce the incidence rate of cardiovascular events.

Design: We analysed retrospectively the data of 27 patients with elevated concentrations of Lp(a) (≥ 60 mg/dl) before and during regular treatment with lipid apheresis. Data of cardiovascular events were collected using self-reporting and medical records for the pre- and during-apheresis treatment periods.

Using these data, the annual incidence rates were calculated.

Results: Patients had incidence rates for cardiovascular events of 1.4 per year before apheresis and 0.1 per year during apheresis.

Regarding only cardiac events, there was a major adverse coronary event rate of 1.4 per year before and 0.08 per year during apheresis.

Conclusion: In this retrospective analysis of data from patients treated with apheresis we could demonstrate that the cardiovascular event rate was significantly reduced after the initiation of apheresis compared to the rates before apheresis. Cardiac events, PCI and CABG frequency were decreased.

At present, lipid apheresis in patients with elevated Lp(a) and progressive cardiovascular disease seems to be a therapeutic option as a last resort.

Memory Stick

GENETIC INTERACTIONS IN THE RENIN-ANGIOTENSIN SYSTEM CONFER INCREASED RISK OF STROKE

Antonela Bazina[1], T. Bozina[2], J. Lovric[2], Z. Poljaković[1], N. Bozina[3,4], J. Sertic[2,3]

[1]Department of Neurology, University Hospital Centre Zagreb, School of Medicine, [2]Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, [3]Department of Laboratory Diagnostics, University Hospital Centre Zagreb, [4]Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia

Background: Stroke is a polygenic and multifactorial atherothrombotic disease. The renin-angiontensin system has an importan role in cerebrovascular disease through a variety of processes. The angiotensin-converting enzyme converts angiotensin I into angiotensin II, which binds the angiotnsin II type-1 receptor and is a potent vasoconstrictor. Both ACE I/D and AT1R A1166C polymorphism lead to an enhanced activity of the angiotensin II-AT1R axis, thereby possibly contributing to circulatory disturbances.

The aim of our study was to investigate ACE I/D and AT1R A1166C polymorphism and the risk of stroke. Methods and materials: Clinical data and genetic and biochemical parameters of 84 patients, aged 30 to 60 years, with acute ischemic stroke were analyzed. A total of 76 controls matched for antropometric parameters, but were carotide ultrasonography alterations-free, older at lest ten years than patients and without history of stroke before age of 70 in two generations. DNA was genotyped for ACE I/D and AT1R A1166C polymorphism.

Result: 51, 45 and 41 % of patients had elevated cholesterol, triglycerides and blood pressure

respectively. The ACE D allele combined with the AT1R 1166C allele did not yield a risk of ischemic stroke. However, the co-occurrence of the homozygous ACE D/D and at least one AT1R 1166C allele was more frequent in the ischemic stroke group of patients under the age of forthy than in the control group (22.4 vs 11%, $p < 0.03$, OR, 2.33; 95% CI, 1.46-3.7).

Conclusion: ACE D/D and AT1R 1166C polymorphism could be risk factor for early occurance of stroke.

Memory Stick

HIGH RESOLUTION MRI BASED-BIOMECHANICAL STRESS ANALYSIS OF CAROTID PLAQUES CAN PREDICT SEVERITY OF WHITE MATTER LESIONS

Umar Sadat, L. Mohsen, V. Young, Z. Teng, J. Gillard

University Department of Radiology, University of Cambridge, Cambridge, UK

Background: There is also sufficient evidence that white matter lesions (WML) are predictive of stroke. Because of its bilateral distribution which does not appear to be completely symmetrical, exploring the relationship of WML with carotid morphology seems justified. Previous studies have explored this relationship providing conflicting results. As there is strong evidence that hypertension has strong association with WML, finite element based- biomechanical stress quantification of plaques provides a unique way of assessing the relationship between carotid plaques and WML because it combines the information about plaque morphology with patient specific blood pressure.

Methods: 40 patients with symptomatic carotid artery disease underwent high resolution magnetic resonance (MR) imaging of carotid plaques in a 1.5 Tesla MR system. The carotid plaque characteristics such as fibrous cap rupture, presence of plaque haemorrhage (PH) etc were identified. Various plaque components such as fibrous tissue, lipid pool and PH were segmented and used for biomechanical simulations. The maximum critical stress (M-CStress) was quantified. WML severity was evaluated using a modified Scheltens score on FLAIR brain imaging. Multiple linear regression model was used to assess the association between modified Scheltens score and various risk factors and plaque characteristics.

Results: Hypertension and M-CStress were found to have significant association with modified Scheltens score ($p= 0.04$ and $p= 0.01$ respectively).

Conclusions: Carotid plaques have strong association with WML severity. Biomechanics-based stress analysis provides a unique method of assessing this relationship as it incorporates information about plaque morphology and blood pressure.

Memory Stick

ASSOCIATION BETWEEN MORPHOLOGICAL CHARACTERISTICS OF ATHEROSCLEROTIC CAROTID PLAQUES AND SUBSEQUENT ISCHAEMIC CEREBROVASCULAR EVENTS IN SYMPTOMATIC PATIENTS WITH MODERATE CAROTID STENOSIS

Umar Sadat, M. Graves, J. Gillard

University Department of Radiology, University of Cambridge, Cambridge, UK

Background: Luminal stenosis of carotid arteries has been used by convention for assessing the severity of carotid artery disease. However, there is sufficient evidence today that morphology of atherosclerotic lesions (plaques) may also be crucial. The association of plaque morphology with subsequent ischaemic cerebrovascular events in symptomatic patients remains unexplored.

Methods: 53 symptomatic patients who had suffered an ischaemic cerebrovascular event underwent high resolution MR imaging of their carotid arteries in a 1.5 Tesla MR system. The carotid plaque characteristics such as fibrous cap (FC) rupture, presence of plaque haemorrhage (PH) etc were identified. These patients were followed up prospectively and the clinical end point for the study was an ischemic cerebrovascular event in the region supplied by the index carotid artery. The association of the time to a cerebrovascular event with MRI-ascertained carotid artery characteristics was analyzed using the Cox regression.

Results: During a median follow-up duration of 338 days, 23% (n=11) patients suffered ischemic cerebrovascular event. FC disruption and PH was associated with development of subsequent cerebrovascular events [Hazard ratio (HR): 7.31 (95% confidence interval (CI) = 0.93-57.27, p=0.05] and [HR: 7.52 (95% CI =0.95-59.06, p=0.05]. Kaplan-Meier plots for the incidence of ipsilateral cerebrovascular events demonstrated that event-free survival was higher among plaques without intraplaque hemorrhage and without FC rupture (p=0.02 and 0.02 respectively).

Conclusions: FC rupture and PH have association with subsequent cerebrovascular events. High resolution carotid MR imaging can identify these features of vulnerable plaques and can be used for risk stratification of such patients.

Memory Stick

HIGH RESOLUTION MRI BASED- BIOMECHANICAL STRESS ANALYSIS OF CAROTID ATHEROMA: A COMPARISON OF TRANSIENT ISCHEMIC ATTACK AND STROKE PATIENT GROUPS

Umar Sadat, Z. Teng, V. Young, J. Gillard

University Department of Radiology, University of Cambridge, Cambridge, UK

Background: Many histological studies have compared symptomatic and asymptomatic atheromatous plaques; however, 'Oxford Plaque Study' is the only histological study which has compared the histological characteristics of carotid plaques responsible for transient ischaemic attack (TIA) and stroke. The biomechanical stress quantification of these two groups however remains undetermined.

Methods: Forty five patients who had suffered a cereberovascular ischaemic event with confirmed underlying carotid artery disease underwent magentic resonance (MR) imaging of their symptomatic carotid artery, within 72 hours of the acute event. MR images were manually segmented for different plaque components and used for finite element analysis. Maximum principle stress of each MR slice was simulated. The maximum critical stress (M-CStress) was used to identify maximum stress at the critical (vulnerable) site of the plaque. M-CStress differences between plaques of TIA and stroke patients were determined.

Results: No M-CStress difference was present between the TIA and stroke patients [258 (191-369kPa) vs. 296(197-512kPa), p=0.15]. Within the TIA group, the Recurrent TIA patients had significantly higher stresses than patients who had suffered a single episode [426kPa (286-582) vs. 250kPa (180-309), p=0.001]. Although the stresses of recurrent TIA patients were higher than stroke patients it was not statistically significant (p=0.64).

Conclusions: Plaques responsible for recurrent TIAs have higher simulated biomechanical stresses than single episode TIA or stroke causing plaques. As there is convincing histological evidence that recurrent TIA plaques have highest inflammatory activity, this study provides biomechanics-based evidence for the first time that high stresses have strong association with enhanced plaque vulnerability.

Memory Stick

IS THERE A CORRELATION BETWEEN CAROTID LUMINAL STENOSIS AND NORMALIZED WALL INDEX? A MAGNETIC RESONANCE IMAGING STUDY

Umar Sadat, Z. Teng, V. Young, J. Gillard

University Department of Radiology, University of Cambridge, Cambridge, UK

Background: Carotid luminal stenosis has been traditionally used for assessment of severity of carotid artery disease. Large multicentre trials such as European Carotid Surgery Trial (ECST) have also used luminal stenosis for calculating the risk of future cerebrovascular ischemic events. However, there is growing evidence that plaque morphology may also be important. Normalized wall index (NWI) is a morphological parameter which indicates the plaque burden and has been identified as a predictor of plaque progression. NWI is calculated by dividing the total wall area by the total vessel area. Higher the NWI, greater is the plaque burden. The two concepts are usually confused to represent the same entity. Strangely enough the relationship between stenosis and NWI also remains unexplored.

Methods: 100 patients with carotid artery disease underwent high resolution magnetic resonance (MR) imaging of carotid plaques in a 1.5 Tesla MR system. The maximum luminal stenosis was calculated using ECST criterion. The axial MR images were segmented to obtain the wall area and total vessel area for NWI calculation. The maximum NWI was then selected for each plaque. Pearson's correlation (r) was then used to assess their relationship.

Results: Luminal stenosis appears to have a weak correlation with NWI ($r^2 = 0.24$) which was significant ($p=0.0001$).

Conclusions: Although apparently NWI and luminal stenosis may appear to represent the same concept, but in reality they are two distinct concepts. This is particularly important to understand because arteries with positive remodelling may not have any luminal stenosis despite high NWI (plaque burden).