Methods: 3T3-L1 cells were treated with cholesterol synthesis inhibitors for 24h. Lipid rafts were isolated by sodium carbonate extraction and sucrose gradient fractionation. Gradient fractions and signal transduction proteins were analyzed by western-blot. Cells in coverslips were fixed and stained for caveolin, plasma membrane cholesterol with filipin, and GM1 with cholera toxin B subunit, and viewed using fluorescence and confocal microscope.

Results: Cholesterol biosynthesis inhibition reduced the activation of signal transduction pathways (ERK1/2, p38 and AKT) in 3T3-L1 cells induced to differentiate. Caveolin was detected in fractions 2-3 of the sucrose gradient fractionation and they were taken as raft-containing fractions. When cells were treated with cholesterol biosynthesis inhibitors, caveolin content in fractions 2-3 were decreased and increased in 9-10. Cholesterol depletion reduced both staining of cholesterol and GM1 and colocalization of those and caveolin and GM1 in plasma membrane.

Conclusions: Cholesterol biosynthesis inhibition disrupts lipid rafts and signal transduction in adipogenesis. This indicates that cholesterol is essential for adipocyte physiology.

PO4-104 HNF4 ALPHA IS ESSENTIAL FOR ACAT2 REGULATION IN HUMAN: LESSON FROM THE MODY PATIENTS

C. Pramfalk[1], E. Karlsson[2], M. Eriksson[3], B. Angelin[3], L. Groop[2], L.L. Rudel[4], P. Parini[1]. *[1]Inst for Laboratory Medicine, KI, Stockholm, Sweden; [2]Dep of Clinical Sciences, Diabetes and Endocrinology, Malmo University Hospital, Malmo, Sweden; [3]Inst for Medicine, Center for Metabolism, Endocrinology and Diabetes, KI, Stockholm, Sweden; [4]Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC, USA*

ACAT2, confined to enterocytes and hepatocytes, is thought to be responsible for cholesteryl ester production in chylomicron and VLDL assembly also in humans. Recently, we identified a liver-specific HNF1 binding site in the human ACAT2 gene and showed that HNF1α and HNF1β, which bind to this site both in vivo and in vitro, is important for the hepatocyte-specific expression of ACAT2. Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of diabetes (NIDDM). Mutations in the HNF1α gene, causes MODY3, and mutations in the HNF4α gene causes MODY1. Thus, we hypothesized that MODY3 and eventually MODY1 (HNF4α is an upstream regulator of HNF1α) patients may have decreased levels of VLDL esterified cholesterol compared to controls. Lipoprotein profiles in serum using a FPLC system showed that MODY1 patients had reduced VLDL and LDL total cholesterol, VLDL esterified cholesterol, and VLDL triglyceride levels compared to controls and MODY3 patients. Moreover, MODY1 patients had reduced LDL esterified cholesterol levels compared to controls. We identified two liver-specific trans-acting HNF4 biding sites in the ACAT2 promoter. Target deletions of these HNF4 binding sites revealed that one of those abolished HNF4α's regulatory effect on the ACAT2 promoter. Our result suggests that the reduced VLDL and LDL esterified cholesterol in MODY1 patients are due to a decreased ACAT2 activity. ACAT2-derived cholesteryl esters have been shown to promote atherosclerosis in different animal models. HNF4α, by regulating the ACAT2 expression, may thus be an important regulator of the development of atherosclerosis.

PO4-105 SAA BLOCKS MODIFIED LDL UPTAKE AND PROMOTES CELLULAR CHOLESTEROL EFFLUX IN A CD36-SPECIFIC MANNER

M.C. De Beer[1], L. Cai[2], A. Ji[2], F.C. De Beer[2], D.R. Van der Westhuyzen[2]. *[1]Department of Physiology, Univ of Kentucky, Lexington, KY, USA; [2]Department of Medicine, Univ of Kentucky, Lexington, KY, USA*

Serum amyloid A (SAA) is an acute phase protein and apolipoprotein of HDL whose expression is markedly up regulated during inflammation and infection. Increasing evidence supports different roles for SAA in lipid metabolism. The scavenger receptor SR-BI is HDL receptor that mediates the selective lipid uptake of HDL-CE by cells. SR-BI also binds and internalizes free and lipid-bound apolipoproteins A-I, A-II and C-III. We and others have reported that SAA is a ligand for SR-BI and promotes cellular cholesterol efflux mediated by both SR-BI and ABCA1. CD36, another prominent class B scavenger receptor, is abundantly expressed in macrophages and may play an important role in lipid uptake and macrophage foam cell formation. Using COS-7 cells that over-express CD36 by adenoviral-mediated gene transfer, we show that that SAA binds to CD36 with high affinity and efficiently inhibits uptake of modified LDL (oxLDL and apoE-/-LDL). SAA also mediates cellular cholesterol efflux in a CD36-dependent manner and is a more efficient acceptor for CD36-dependent cholesterol efflux than apoA-I. The CD36-dependent efflux to SAA and inhibition of modified LDL uptake was confirmed in peritoneal macrophages lacking this receptor. Furthermore, SAA was able to remove cholesterol from peritoneal macrophages lipid-loaded by AcLDL. We conclude that the acute phase protein SAA may play an important role in regulating cellular cholesterol flux.

PO4-106 GLYCOXIDATION OF LOW-DENSITY LIPOPROTEIN ACTIVATES MAPK-ERK/JNK PATHWAYS AND AP-1 COMPLEX

F. de Nigris, C. Schiano, C. Fiorito, C. Maione, M. Rienzo, A. Casamassimi, F. Muto, N. Cirillo, M. Policastro, F. Gombos, G. Fatigati, A. Lanza, A. Palagiano, V. Sica, C. Napoli. *Department of General Pathology, Division of Clinical Pathology and Excellence Research Center On Cardiovascular Diseases, and Regional Center MRI, 1st School of Medicine, II University of Naples, Naples, Italy*

There is evidence of glycoxidized LDL (glc-oxLDL) in plaques. The goal of the study was to explore mechanisms by which glcLDL and glc-oxLDL modulate MAPK-ERK and JNK activities, and AP-1 complex in human coronary SMC. The percentage of MAPK activity induced by glc-oxLDL was about 30%, while glcLDL induced 5%. In the presence of PD98059, MAPK activity at 24hours was reduced to 48%. Blot analysis revealed a marked increase in phosphorylated form of c-Jun in SMC after incubation with glcLDL and glc-oxLDL, compared to native LDL and oxLDL. GlcLDL and glc-oxLDL significantly increased JNK activity (2.54±2 and 93.1±5 arbitrary units, respectively;n=4), compared to native and oxLDL (2.0±0.8 and 83±0.5 arbitrary units; $p < 0.01$ for both comparisons). We found a marked AP-1 activation in nuclei of cells exposed to glc-oxLDL. AP-1 complex was subjected to dissociation analysis, to determine its DNA stability at the site of AP-1 binding. AP-1 complex in cells exposed to glc-oxLDL exhibited a slower dissociation with a complex still bound after 10min, while the complex was dissociated within 2min in oxLDL-treated cells. Thus, AP-1 complex in glc-oxLDL-treated cells binds the DNA in a stable fashion than that achieved with oxLDL. There was a supershifted band in the presence of antibodies against c-Jun/JunB/Fra-1. Glc-oxLDL-treated cells mainly supershifted with c-Jun/Fra-1 antibodies. AP-1 complex composition is different in oxLDL and glc-oxLDL, and could be responsible for differential stability of the two complexes. Thus, glc-oxLDL stimulates MAPK-ERK and JNK, coupled to activation of the c-Jun and Fra-1 elements of AP-1 complex.

PO5 MOLECULAR GENETICS AND GENE–ENVIRONMENT INTERACTION

PO5-107 THE INTERACTION BETWEEN ADHERENCE TO THE MEDITERRANEAN DIET AND METHYLENETETRAHYDROFOLATE REDUCTASE C677T MUTATION ON HOMOCYSTEINE CONCENTRATIONS

G. Dedoussis[1], D.B. Panagiotakos[1], C. Pitsavos[2], C. Chrysohoou[2], C. Stefanadis[2]. *[1]Harokopio University, Athens, Greece; [2]Athens Medical School, Athens, Greece*

Background: It has been suggested that dietary and genetic factors may influence the effect of raised homocysteine concentrations on coronary heart disease risk. We evaluated the association of the interaction between adoption of Mediterranean diet and Methylenetetrahydrofolate Reductase (MTHFR) C677T mutation on homocysteine concentrations, in healthy adults from the ATTICA study.

Methods: We studied demographic, lifestyle, clinical, biochemical and genetic information from 322 men (46 ± 13 years) and 252 women (45 ± 14 years), without any clinical evidence of cardiovascular or any other chronic disease. We also measured total plasma homocysteine concentrations, the distribution of MTHFR genotype and adherence to the Mediterranean diet.

Results: The distribution of MTHFR genotypes was: homozygous normal (CC) genotype, 41%; heterozygous (CT), 48%; and homozygous

POSTER SESSIONS

mutant (TT) genotype, 11%. Homocysteine concentrations were higher in TT as compared to CC and CT (15.8 ± 9 vs. 11.3 ± 8 vs. 10.8 ± 9 μmol/L, p < 0.001). The Mediterranean diet score was not associated with homocysteine (p = 0.89). However, stratified analysis revealed that adherence to Mediterranean diet was associated with reduced homocysteine in TT and CT (Beta = -0.21, p = 0.002 and Beta = -0.14, p = 0.025, respectively), but not in CC individuals (Beta = -0.03, p = 0.38), after controlling for potential confounders.

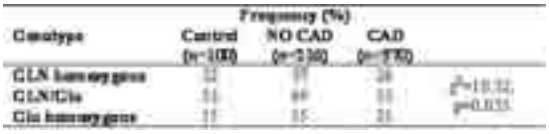
Conclusion: The observed effect of MTHFR C677T gene – diet interaction on homocysteine concentrations may provide a pathophysiological explanation by which Mediterranean diet may influence coronary risk in people with increased homocysteine concentrations.

PO5-108 GLU-27 VARIANT OF BETA 2-ADRENERGIC RECEPTOR POLYMORPHISMS IS AN INDEPENDENT RISK FACTOR FOR CORONARY ATHEROSCLEROTIC DISEASE

E. Barbato, A. Berger, L. Delrue, F. Van Durme, G. Manoharan, T. Boussy, G.R. Heyndricx, B. De Bruyne, M. Vanderheyden, W. Wijns, J. Bartunek. *Cardiovascular Center OLV Ziekenhuis, Aalst, Belgium*

Glu-27 variant of β2-adrenergic receptor (β2-AR) polymorphism has been associated to risk factors for coronary atherosclerotic disease (CAD) like obesity, dyslipidemia and hypertension. Conflicting data have been reported concerning their influence on CAD and cardiovascular events. Aim of the study was to investigate whether (a) β2AR polymorphisms are associated with CAD; and (b) the potential impact, if any, of these polymorphisms on cardiovascular clinical events in patients presenting with angina-like pain or silent ischemia.

We screened 786 consecutive patients referred to cardiac catheterization because of angina-like pain or silent ischemia for Gln27Glu β2AR polymorphism. Patients were divided in 2 groups according to the presence (epicardial stenosis > 30%) or absence of CAD at the angiography. Hundred subjects from blood donor center served as controls. Clinical endpoints were evaluated at baseline and up to 6 years follow-up. Glu-27 homozygous genotype and Glu-27 allele (Glu-27, allele frequency: 47% CAD vs. 39% NO CAD, p<0.05) were more frequent in patients with CAD. At multivariate analysis, patients carrying Glu-27 allele showed a significantly higher risk of developing CAD (O.R.: 1.78, 95% C.I.: 1.21-2.63, p=0.004). At clinical follow-up, a higher incidence of coronary revascularization was noted in Glu-27 homozygotes as compared with Gln-27 homozygote patients.



In patients at high risk for CAD and/or angina-like pain, Glu-27 allele of β2 adrenergic receptor polymorphism is an independent risk factor for CAD and appears to be associated with higher incidence of myocardial revascularization.

PO5-109 IDENTIFICATION OF TWO NEW LARGE DELETIONS IN THE LOW DENSITY LIPOPROTEIN RECEPTOR (LDLR) GENE IN THE FRENCH CANADIAN POPULATION

L. Bernier, L. Boulet, M. Roy, R. Dufour, J. Davignon. *Institut de Recherches Cliniques de Montreal, Montreal, QC, Canada*

Backgrounds: In the French Canadian population six mutations appear to be responsible for about 85% of FH cases. Two of these mutations are large deletions. The most prevalent deletion is a >15kb deletion of the promoter and first exon; the second, a 5kb deletion that removes exons 2 and 3. The high frequency of these deletions has been attributed to a founder effect. Other mutations are present in the population but at a much lower prevalence.

Methods: Southern blotting and a PCR-based method were used for routine screening of hyperlipidemic patients from our Lipid Clinic. Two new deletions were identified because of an unusual pattern of band migration on Southern blots. RFLP analysis using various restriction enzymes was used to further characterized the deletions. Identification of the deleted exons was done by Semi-quantitative Real Time PCR. Deletions borders were identified by sequencing.

Results: We recently identified patients who were heterozygous for two large, previously unreported deletions. The first deletion covers 3813 bp and removes exons 7 and 8. The second covers 5994 bp and removes exons 3-6. In both cases the deletion was also found in other members of the family and segregated with high LDL cholesterol levels.

Conclusions: This study serves as a reminder to be cautious when screening FH patients. In addition to the very convenient PCR-based methods, other techniques (such as RFLP) should remain on hand to test FH patients who appear normal using the new tests.

PO5-110 DEVELOPING ASSAYS FOR COMMON RUSSIAN LDL RECEPTOR GENE MUTATIONS AND FOR GENE EXPRESSION BY MEANS OF REAL-TIME PCR

M.Y. Mandelshtam[1], S.N. Pchelina[2], Y.A. Tatishcheva[1], B.M. Lipovetsky[3], V.O. Konstantinov[4], A.D. Denisenko[5], M.V. Dubina[2], V.B. Vasilyev[1]. *[1]Department of Molecular Genetics, Institute of Experimental Medicine, St.Petersburg, Russia; [2]St.Petersburg State Medical University, St.Petersburg, Russia; [3]Institute of Human Brain, St.Petersburg, Russia; [4]St.Petersburg State Mechnikov Medical Academy, St.Petersburg, Russia; [5]Department of Biochemistry, Institute of Experimental Medicine, St.Petersburg, Russia*

Background and Aims: Familial hypercholesterolemia (FH) is a common monogenic disorder caused by LDL receptor gene mutations. Study of 74 probands with FH from St.Petersburg allowed identification of 30 disease-causing mutations in 41 families. Out of these mutations most common were G197del (c.652del3) deletion responsible for 30% (7 out of 22) of FH cases in Ashkenazi patients and C139G (c.478 T>G) mutation found in four Slavic families out of St.Petersburg, Moscow and Novosibirsk.

Methods: Real-time PCR technology was used to develop rapid screening tests for recurrent mutations. Two specific TaqMan fluorescent probes were developed to discriminate between G197del mutant and normal alleles with the same flanking primers. Different approach was used to test for C139G mutation caused by nucleotide substitution: in this case ARMS technology was applied, with the same fluorescent TaqMan probe for both alleles, but with mutation-specific flanking primer.

Results: Since large variety of mutations causes FH in Russia and many patients bear unknown mutations, gene expression assay for LDL receptor gene may be in many cases more informative for diagnosis compared to specific mutation testing. To study this question we have used real-time PCR approach with probes developed to measure LDR receptor gene mRNA level in FH patients peripheral blood monocytes. Most reliable results were obtained when using ubiquitin (UBIC) mRNA rather than beta-actin or GAPD for normalization of results.

Conclusions: Our data demonstrate that real-time PCR may be a potent method for FH diagnostics.

Current research was supported by RFBR grant No. 05-04-48235.

PO5-111 C242T POLYMORPHISM OF NADPH OXIDASE P22PHOX GENE PREDICTS MAJOR CARDIOVASCULAR EVENTS (MACE) IN HIGH-RISK INDIVIDUALS

B. Conti[1], A. Montali[1], S. Valiante[1], C. Gaudio[2], G. Panetta[2], A. Pergolini[2], R. Antonini[1], F. Violi[3], F. Campagna[1], M. Arca[1]. *[1]Department of Clinical and Applied Medical Therapy, I Medical School, University La Sapienza, Policlinico Umberto I, Rome, Italy; [2]Department of The Heart and Great Vessels Attilio Reale, I Medical School, University La Sapienza, Policlinico Umberto I, Rome, Italy; [3]Department of Experimental Medicine and Pathology, I Medical School, University La Sapienza, Policlinico Umberto I, Rome, Italy*

Background and purpose: NADPH oxidase is the key enzyme for superoxide production in the vascular system. A negative association between the functional C242T single-nucleotide polymorphism (SNP) in the gene coding for p22phox, a major component of NADPH oxidase, and the presence of atherosclerosis has been reported in cross sectional studies. The impact of this SNP on prospective cardiovascular risk has never been evaluated.

Methods: Among 534 patients with coronary stenosis (>50% in at least 1 vessel) examined between 1994-1998, follow-up data were obtained in 286 patients (239 men, 47 women, aging 59.3±8.9 years). Major