SYNERGISTIC EFFECTS OF HEPATIC LIPASE (HL) PROMOTER AND CETP PROMOTER POLYMORPHISM ON INCREASED HDL-C LEVELS IN WOMEN

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Backgrounds: CETP promoter –1337 C/T polymorphism has stronger association with low plasma CETP levels than TaqIB2 in the Japanese population. Molecular basis of mild hyperalphalipoproteinemia is not well known except heterozygous CETP deficiency.

Methods: We investigated the synergistic effects of HL –514 C/T and CETP promoter –1337 C/T polymorphism on lipid profiles, plasma CETP mass, and HL activities in 236 subjects (male n=87) who were examined post-heparin lipase activities 10 minutes after intravenous injection of 30 units of heparin per kilogram of body weight.

Results: There was a significant inverse dose-dependent association between the number of HL –514 T allele and HL activity especially in women ($p<0.05$). CETP T allele was associated with lower CETP concentrations, however the difference of which did not reach statistical significance(-10.5%, p=0.17 in women and -3.9%, p=0.58 in men). Both HL –514 C/T and CETP –1337 C/T polymorphism were associated with HDL-C levels, especially between HL –514CC/ CETP –1337 CC genotype and HL –514 TT/CETP –1337 CT or TT genotype (67±4 vs 51±3 mg/dl in women, 50±4 vs 41±2 mg/dl in men, $p<0.05$). The frequency of the combined HL –514 TT/CETP –1337 CT or TT genotype was found in 10 % in both gender.

Conclusion: The combined reduction of HL and CETP explains a significant fraction of mild hyperalphalipoproteinemia in women.

LIPOPROTEIN LIPASE ACTIVITY CORRELATED POSITIVELY AND HEPATIC LIPASE ACTIVITY INVERSELY WITH SERUM ADIPONECTIN LEVELS IN JAPANESE HYPERLIPIDEMIC MEN

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Aims. To understand the potential mechanisms for dyslipidemia in low adiponectin, we investigated the association of lipoprotein lipase (LPL) and hepatic triacylglycerol lipase (HTGL) activities with serum adiponectin levels. Methods. Fifty-five hyperlipidemic Japanese men were recruited for this study. LPL and HTGL activities in post-heparin plasma (PHP) were measured using Triton X-100 emulsified-[14C] triolein. LPL mass in PHP was measured using anti-human LPL monoclonal antibody. Serum adiponectin levels were determined by an enzyme-linked immunosorbent assay system. Results. LPL activity had a positive relationship with LPL mass ($r=0.643$ $p<0.0001$). LPL activity had a positive relationship with HDL2, but had no relation with HDL3, while HTGL had positive relationship with HDL3, but had no relationship with HDL2. LPL activity had a positive($r= 0.345$, $p=0.0099$) and HTGL activity had an inverse ($r=-0.365$ $p=0.0062$) relationships to adiponectin levels. Adiponectin levels had an inverse relation with BMI ($r=-0.327$ $p=0.014$), fasting insulin ($r=-0.409$, $p=0.007$) and TG levels ($r=-0.378$ $p=0.044$), and had a positive relation with HDL2-C($r=0.378$ $p=0.0044$) but not with HDL3-C. Conclusion. LPL and HTGL may have been regulated by adiponectin in opposite direction.

LIPOPROTEIN(a) LEVELS, APOLIPOPROTEIN E4 AND HEPATIC LIPASE PROMOTER GENE POLYMORPHISM (-514C/T) IN FAMILIAL HYPERCHOLESTEROLEMIA

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Familial hypercholesterolemia (FH) carries a marked increased risk of coronary heart disease (CHD). However, there is considerable variation between individuals in susceptibility to CHD indicating the possible influence of environmental and genetic variations other than LDL receptor on lipid and lipoprotein phenotypes and thereby the risk of CHD. Common variations in the genes coding for proteins involved in lipoprotein metabolism have been investigated on an FH background. In this study we estimated the levels of lipoprotein(a) [Lp(a)] and evaluated the possible influence of apolipoprotein (apo) E gene polymorphism (E2, E3, and E4) and hepatic lipase (LIPC) promoter gene polymorphism (-514C/T) on lipid and lipoprotein levels in 55 unrelated and related patients with clinical features of possible heterozygous FH and 76 normolipemic healthy controls. Lp(a) levels were estimated by ELISA, apo E genotyping was done by ARMS-PCR and LIPC genotyping by PCR-based restriction enzyme analysis. Median Lp(a) levels were significantly elevated in patients (36 mg/dl) then in normolipemic controls (17.2 mg/dl). The E4 allele frequency was significantly higher in patients (0.21) than in controls (0.05). The patient group was divided into two: with and without E4. The total cholesterol, triglyceride, apo A1 and apo B levels were significantly higher in the E4-present group but the difference for LDL cholesterol and Lp(a) levels were non-significant. The frequency of the rare 'T' allele of the LIPC gene was found to be 0.16 and 0.22 in the control and patient group respectively (non-significant). The polymorphism was associated with apo A1 levels in the patient group without allelic effect. No other influence was observed. In conclusion, the study supports the concept that elevated Lp(a) and apo E4 genotype may add to the risk of CHD in FH.

INCREASED ARTERIAL WALL THICKNESS IN LPL DEFICIENT PATIENTS

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Lipoprotein Lipase (LPL) deficiency, a rare disorder, is due to the absence of LPL activity. Consequently, chylomicron clearance from plasma is delayed, leading to increased triglyceride (TG) serum levels. Hypertriglyceridaemia is a risk factor for atherosclerosis. Reports on LPL deficiency, based on small numbers of subjects, show conflicting results on morbidity and mortality; the effect of the disorder on atherosclerosis progression is unknown. We investigated arterial walls of carotid and femoral ultrasound intima-media thickness (IMT) measurements. IMT is considered a validated surrogate endpoint for atherosclerosis and atherosclerotic disease risk.

In a cross-sectional study, clinical, laboratory and IMT measurement data of 16 LPL deficient subjects (age 38.8(SD12.9) range16.4-69.8 years), BMI 21.9(2.6)kg/m^2 and TG 31.4(11.6)mmol/l) were compared to 50 healthy volunteers (age 32.3(SD12.0) range 23.0-61.4 years), BMI 23.3(3.1)kg/m^2, TG 1.0(0.6)mmol/l). Estimated IMT increase with age was analysed by descriptive statistics and curve fit models.

In the LPL-deficient population IMT was increased: 0.56 (SD0.05) versus 0.50 (SD0.06mm) in controls; δ0.064(SED0.020)mm (p=0.009). Regression analyses indicated significant IMT increase with age in patients (p=0.001) and controls (p=0.025), as well as between the groups (p=0.01).

These analyses indicate a more rapid progression of IMT in LPL-deficient individuals. Consequently LPL-deficient individuals may be at higher cardiovascular risk when compared to unaffected controls despite extremely low LDL concentrations.

STATINS ENHANCED LIPOPROTEIN LIPASE EXPRESSION IN 3T3-L1 PREADIPOCYTES

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It is known that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) enhance low-density lipoprotein (LDL) receptor and lower LDL cholesterol in the blood. But, triglyceride (TG)-lowering effect is also observed during statins administration. To clarify the possibility that statins enhance LPL activity and its mechanism, the effects of statins on the expression of LPL in adipocytes were studied. When statins (pravastatin, simvastatin, atorvastatin and pitavastatin) were added to the culture medium of mouse 3T3-L1 preadipocytes with final concentrations of 1 μM for 3 days, LPL activity increased at various extent. Among those satins, pitavastatin increased LPL activity most strongly. Western and Northern blotting analysis showed that LPL protein and m-RNA were strongly expressed by the addition of pitavastatin. By the addition of mevalonate (10 μM, 3 days), LPL activity reduced significantly From these results, statins, especially pitavastatin increased the LPL expression in 3T3-L1 preadipocytes. These results suggested that TG-lowering effect of pitavastatin might be mediated by enhancement of LPL production in adipocytes.

THE ANGIOTENSIN II RECEPTOR ANTAGONIST VALSARTAN IMPROVED LDL PARTICLE SIZE IN PATIENTS WITH HYPERTENSION

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There exists lipoprotein lipase mass in preheparin serum (preheparin LPL mass), even though the activity is scarcely found. We reported that preheparin LPL mass could reflect some of the LPL production in a whole body, and might be related to insulin sensitivity [1]. The angiotensin II receptor antagonists are reported to improve the lipid metabolism and the insulin resistance. The present study evaluated the effect of the angiotensin II receptor antagonist, valsartan, on the lipid profile and preheparin LPL mass in patients with hypertension. We treated 30 patients with valsartan 80mg orally once daily for 12 weeks. We studied the changes in blood pressure, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), LDL particle size and preheparin LPL mass. LDL particle size was decided by relative mobility value of peak of LDL fraction (LDL-Rm) detected by lipoprotein polyacrylamide gel disc electrophoresis. Preheparin LPL mass was measured by the sandwich enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibody (Daiichi pure chemicals, Japan). Valsartan significantly lowered systolic blood pressure by 17.9±17.3 mmHg and diastolic blood pressure by 11.2±11.3 mmHg. There were significant decrease in levels of TC (-15.2±13.8 mg/dl), TG (-29.0±60.6 mg/dl), LDL-C (-11.0±10.3 mg/dl), and LDL-Rm (-0.036±0.023). Preheparin LPL mass levels were significantly increased. To clarify the mechanism by which valsartan decreased LDL-Rm, we observed the correlation between LDL-Rm and various factors. LDL-Rm was negatively correlated with preheparin LPL mass. These results suggested that valsartan might improve lipid metabolism and insulin sensitivity, and the drug might enlarge LDL particle size by increasing of preheparin LPL mass.

1) Totsuka et al. Atheroscler 2000; 153: 175-179.

EFFECT OF CURCUMIN AND ESCULETIN ON THE EXPRESSION OF MUTATED LIPOPROTEIN LIPASE

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Lipoprotein lipase (LPL) catalyzed the hydrolysis of triglycerides in very low-density lipoprotein and chylomicrons. The defect in LPL causes type I hyperlipoproteinemia. In our previous study, L252V and L252R mutants were found in type I hyperlipoproteinemic patients. The aim of this study was to evaluate the effect of curcumin and esculetin on these mutated LPL. HEK293 cells were transfected with wild type and mutated LPL, respectively. These transfected cells were then treated with curcumin and esculetin, respectively. Neither curcumin nor esculetin increased the mRNA expression of wild type and mutated LPL. However, curcumin increased 7-fold L252R LPL activity and 5.7-fold in mass in culture medium, and LPL activity and mass of L252V transfected cells increased 2.5-fold and 2.3-fold, respectively. Contrary to the culture medium, both activity and mass were decreased in wild type and mutants in cell lysate. The effect of esculetin on wild type and mutated LPL activity and mass both in culture medium and cell lysate was the same as that of curcumin. This suggests that the mechanism of both curcumin and esculetin for these effects is the same, and the clinical application remain to be determined.

***IN SILICO* IDENTIFICATION OF LIPOLYTIC ENZYMES AND THEIR POTENTIAL ROLE IN THE MOBILIZATION OF CHOLESTEROL**

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Many lipolytic enzymes belong to the large and structurally diverse family of α/β-hydrolases. Members of this family share a characteristic mechanism of the catalytic action. They possess a highly conserved core structure which accomodate the catalytic triade (a serine, an aspertate and a histidine). In spit of the wealth of lipolytic enzymes, there is evidence suggesting that additional lipolytic activities exist. Our group is specifically interested in the lipid metabolism of murine macrophages and foam cells. We aim at identifying yet unknown genes that are involved in the homeostasis of cholesterol.

Murine proteins with suggested lipolytic function were identified by advanced computational analysis. Tissue expression pattern of 14 potential lipase/esterase candidates were determined. Targets which were found to be expressed in murine macrophages and/or foam cells were further characterized. Full-length cDNAs of these candidates were cloned into eukaryotic expression vectors. The simian kidney cos7 cell line was transfected with the constructs of hypothetical hydrolases. Triglyceride lipase and cholesteryl ester hydrolase activities were assessed. The part of our project was focussed in particular on the determination of cholesteryl ester hydrolase activity as accumulation of cholesterol esters in macrophages are a key event in foam cell formation. The results of this ongoing work will be presented at the meeting.

HTS ASSAY DEVELOPMENT FOR CARDIOVASCULAR-RELEVANT LIPASES

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Plasma levels of high-density lipoproteins (HDL) cholesterol are strongly inversely associated with atherosclerotic cardiovascular disease, and overexpression of HDL proteins reduces progression and even induces regression of atherosclerosis. Therefore, HDL metabolism is recognized as a potential target for therapeutic intervention of atherosclerotic vascular diseases. The molecular regulation of HDL metabolism is influenced by several extracellular lipases. Among them hepatic lipase (HL) and secretory phospholipase A2 (sPLA2) play a key role: HL hydrolyzes HDL triglyceride and phospholipids, generating smaller lipid-depleted HDL particles, while sPLA2 is an acute-phase protein that exhibits phospholipase activity and during acute and chronic inflammatory states such as in atherosclerosis could contribute to the development and progression of atherosclerotic lesions.

Here, we describe the recombinant expression in an eukaryotic system of the human secretory phospholipase A2 and of the human hepatic lipase; both enzymes were purified in order to configure HTS homogenous assay for identifing enzyme inhibitors.

A fluorescence-based HTS assay was established for both purified proteins: the assay conditions were optimized in order to increase activity and the kinetic parameters were determined:

- sPLA2 assay is based on the ability of the enzyme to hydrolyse glycerophospholipids releasing free fatty acids and lysophospholipids. In this assay the sPLA2 hydrolysis releases a fluorophore covalently linked to a fatty acid, so the sPLA2 activity is followed by monitoring the increase in time of fluorescence intensity; a putative sPLA2-specific inhibitor would be identified by a reduced fluorescent signal.
- HL assay is based firstly on its triacylglycerol lipase activity with internally quenched fluorescent substrates that release pyrene fluorophores; therefore an increase in fluorescence intensity correlated to enzyme activity; also in this case HL- specific inhibitor would be identified by a reduced fluorescent signal.

LDL APHERESIS AS A POTENTIAL THERAPEUTIC APPROACH IN PREECLAMPSIA– A PREGNANCY-RELATED HYPERTENSIVE DISORDER

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Preeclampsia (PE) is a multiorgan disease characterised by hypertensive glomerular dysfunction, proteinuria and coagulatory abnormalities. It complicates 2-4% of pregnancies and is a major cause of maternal and fetal morbidity and mortality. The pathogenesis of PE is still not known. It is generally accepted that endothelial dysfunction plays a central role. Immediate cessation of pregnancy remains the only effective treatment. Alterations of lipoprotein concentration, procoagulatory and proinflammatory markers have been associated with endothelial dysfunction in PE. Heparin-mediated extracorporeal LDL precipitation (H.E.L.P.) apheresis efficiently removes plasma LDL, Lp(a), fibrinogen and hs-CRP in patients with CHD, familial hypercholesterolemia, and after heart transplantation. We recently showed it also reduces circulating levels of AMs, MCP-1, ET-1, LBP, TF, and homocysteine. Since PE and CHD share some common risk factors, we treated preeclamptic women with H.E.L.P. apheresis. We enrolled 10 PE patients (32.2±5.8 years, gestational age 26.6±2.5 weeks). Six of 10 patients were treated repeatedly with H.E.L.P. Number of therapies in the 6 patients varied from 1 to 7. Gestational age was prolonged by 5, 23, 17, 23, 3 and 19 days respectively. Five newborns from the preeclamptic mothers on LDL apheresis showed normal growth and development. One extremely preterm baby died of sepsis in neonatal stage. Newborns (n=3) from the untreated mothers suffered from serious complications during neonatal stage and one died immediately after birth. Therapy was well-tolerated by PE patients. It reduced plasma TG, LDL-C, VLDL-C, Lp(a), fibrinogen and hs-CRP on average by 40%, 45%, 42%, 53%, 53%, 47% respectively. It also reduced circulating levels of TNFα, VCAM-1, LBP by 41%, 23%, 14% respectively. Plasma viscosity was reduced on average by 12% ($P<0.001$). Doppler ultrasound examination showed improved placentofetal blood flow. Activation of transcription factors NF-κB and AP-1 which play a central role in inflammatory processes was reduced both in maternal PBMC and placental tissue in H.E.L.P.-treated patients than untreated. Even though the number of patients was small, we conclude LDL apheresis in management of PE deserves consideration.

LECITHIN:CHOLESTEROL ACYLTRANSFERASE (LCAT) DEFICIENCY AND FISH EYE DISEASE IN ITALY

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Mutations in the LCAT gene lead to two rare autosomal diseases: familial LCAT deficiency (FLD), and fish-eye disease (FED). In FLD homozygotes, LCAT is either absent or lacks catalytic activity; as a result, there is very little cholesteryl esters in plasma. In FED homozygotes, LCAT does not esterify cholesterol in HDL, but esterifies cholesterol in VLDL and LDL; subnormal cholesteryl esters levels are present in plasma. Clinically, FLD homozygotes present with corneal opacity, anemia, proteinuria, haematuria, and ultimately renal failure, often requiring kidney transplantation. In contrast, FED homozygotes do not appear to have typical clinical manifestations except for corneal opacification. Cardiovascular risk associated to these two different diseases is still not clarified. Recently, we have identified 13 probands with mutations in the LCAT gene; the genetic analysis identified 17 mutations, 15 novel and 2 already described. Seven homozygous probands have FLD and present with corneal opacity and anemia; kidney disease was found in 6 of them, two subjects underwent renal transplantation. Plasma HDL-C concentration is markedly reduced (8.7±5.2 mg/dl), as well as plasma apoA-I and apoA-II levels (37.9±2.2 and 7.7±1.2 mg/dl, respectively). Plasma LCAT activity and cholesterol esterification rate are nearly zero, and LCAT mass is significantly reduced (1.3±0.2 μg/ml, n.v. 3.1-6.7 μg/ml). Three homozygous probands have FED and present with corneal opacity; one has proteinuria. Plasma HDL-C, apoA-I, and apoA-II levels are markedly reduced (12.5±4.9 mg/dl, 51.3±10.4 mg/dl, and 12.8±1.8 mg/dl, respectively). Cholesterol esterification rate is normal (40.5±2.5 nmol/ml/h, n.v. 30-60 nmol/ml/h), while plasma LCAT activity is nearly zero. LCAT mass is almost half of control values (2.18±0.37 μg/ml). None of the probands suffered from premature cardiovascular disease. Three of the identified probands are heterozygotes; at the moment it is not possible to classify them as FLD or FED. Twenty-nine heterozygotes (22 FLD and 7 FED) were identified among the probands' relatives.

EFFECTS OF HEPARIN-MEDIATED EXTRACORPOREAL LOW-DENSITY LIPOPROTEIN PRECIPITATION BEYOND LOWERING PROATHEROGENIC LIPOPROTEINS-REDUCTION OF CIRCULATING PROINFLAMMATORY AND PROCOAGULATORY MARKERS

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In addition to hypercholesterolemia, proinflammatory and prothrombotic markers have been suggested to play an important role in atherogenesis. We examined whether heparin-mediated extracorporeal low-density lipoprotein precipitation (HELP) therapy modulated the circulating levels of proinflammatory and prothrombotic markers. Twenty-two coronary heart disease (CHD) patients undergoing regular HELP-apheresis (18 males, 4 females, mean age 57.3 ± 10.9 years) were enrolled in this study. A single HELP therapy treatment significantly decreased the circulating levels of high sensitivity C-reactive protein (hs-CRP), soluble vascular adhesion molecule-1 (sVCAM-1), soluble E-selectin, lipopolysaccharide binding protein (LBP), endothelin-1(ET-1) and monocyte chemoattractant protein-1 (MCP-1) on average by 67, 37, 24, 27, 24 and 15%, respectively. Prothrombotic factors including fibrinogen, tissue factor (TF), soluble CD40 ligand (sCD40L), and homocysteine were decreased by 66, 27, 16, and 22%, respectively. In accordance with previous studies HELP therapy reduced total cholesterol, low density lipoprotein (LDL) cholesterol, and Lp(a) mass by 50, 61, and 62%, respectively. Our data suggest that simultaneous reduction of proinflammatory and prothrombotic factors together with atherogenic lipoproteins by HELP-apheresis may contribute to improvement of endothelial dysfunction and thereby inhibit progression of atherosclerotic lesions and stabilize the existing plaque.

LONG-TERM LIPOPROTEIN (A) MODIFICATION IN FH PATIENTS TREATED WITH LDL-APHERESIS

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AIM: The evaluation of long-term Lp(a) treatment of FH patients with two different LDL-apheresis techniques: DALI and secondary filtration (SF).
METHODS: 7 patients have been evaluated, 5 male and 2 female, 53± 4 years old, affected by heterozygous FH. 3 patients have been treated with DALI technique, while 4 patients with secondary filtration technique. Before and after LDL apheresis, patients were subjected to venous blood samples sampling in order to evaluate ematochemical change, especially as it regards Lp(a), TC, LDL-C, HDL-C, TG.
RESULTS: TC and LDL-C in pre- and post-apheeresis (DALI + FS) undergo a very important decrease both basic than after one year ($p<0.01$). Even if Lp(a) decreases, in basic for –69% and after one year in –63,8%, it doesn't get to a statistic importance. CT and LDL-C significantly decrease with DALI system ($p<0.001$), while Lp(a), TG and HDL-C, are reduced, but not in as significant way (presumably because of the large SD of these parameters). After one year of SF decrease in TC and LDL-C are both less than those obtained with DALY SF ($p<0,01$). Lp(a) and TG reduction are less intensive with SF, contrarily to HDL-C that is less influenced by the DALI than the SF (-11,8% vs. -26%). Percentual Lp(a) change in basal and after 1 year pre-apheresis was a 5,9% increase in SF, and a 8,6% decrease in DALI.
CONCLUSIONS: our results show that, after the first apheretic sessions, with the two methods used, DALI and FS, LDL-C and Lp(a) basic values have an evident decrease. DALI technique, after one year, produces significant Lp(a) , CT, LDL-C values decreases. SF induces a light increase of Lp(a). HDL-C level decreases above all after SF, but after a treatment of one year, Lp(a) values in pre-apheresis increases. The two techniques can reduce plasma level of atherogenic lipids in an effective way, even if the DALI capacity of removal is upper than that of SF one.

LOW LEVEL LAZER THERAPY AND LIPID METABOLISM

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Though lipid metabolism is considered to be important mostly in the pathology of the organism (atherosclerosis, ischemic heart disease, obesity) is the main and the only metabolism in the organism. Lipid is created in the organism recovery system in a certain part of liver on the basis of some highly active microelements, i.e. pi-meson with the rest mass of 139.5673 eV. Red and infrared radiation are considered to exert certain energizing influence upon the organism, i.e. higher temperature in those skin layers that absorb this radiation, and it is absorbed mainly in derma, but about 30% penetrates 40 mm deep and reaches subcutaneous fat and organs under it. It mostly refers to infrared radiation. Radiation quanta carry too little energy to cause photo-chemical action though it is not possible to absolutely exclude such effect for 0.63 mkm-1.3 mkm spectral interval. It is our analysis that any low-intensity laser radiation is represented by quantum or an energy-carrier particle typical for a certain wave length and its mass equals the mass of a pi-meson – lipid, which not only energizes but is introduced as a fresh microelement instead of a used one. Two classes of lipoproteins are most important for cholesterol transportation – HDL that transport cholesterol out of cells and LDLP that transport cholesterol into cells. In healthy humans we observe parallelism between the amount of total cholesterin (TC) in blood plasma and TC amounts included into LDL. Similar parallelism exists also between triglycerides (TG) amount in blood plasma and VLDL.We examined 60-75-year-old patients: in 28 patients drug therapy was combined with laser therapy and a control group of 13 patients with only drug therapy. All patients were hospitalized in acute conditions of different gastro-intestinal diseases. Polymorbism was observed in all cases. TG in the observed group at the time of admission was normal in 60.71 % of patients, after treatment – in 64.28%. In the control group it lowered from 53.8% in the norm to 23.07%.

CHANGES IN SERUM LIPID PROFILE BEFORE AND AFTER INTERFERON TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C

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Backgrounds: An impaired lipid metabolism is often found in patients with chronic liver diseases. Infection with hepatitis C virus (HCV) is reported to be associated with serum hypocholesterolemia. Interferon (IFN) has been shown to eliminate HCV viremia and to reduce serum alanine aminotransferase (ALT) in patients with chronic hepatitis C.
Objectives: We conducted a longitudinal study to characterize the serum lipid profile in Japanese patients with chronic hepatitis C and to investigate the changes before and after IFN treatment.
Methods: A total of 89 consecutive chronic hepatitis C patients (55 males, mean age 57.5 ± 10.9 years; 34 females, mean age 59.5 ± 7.4 years) were studied to assess the following: (1) HCV genotype, viral load, hepatic histology, ALT, sex, age, and body mass index (BMI) on lipid profile before IFN; (2) whether lipid parameters could predict response to IFN treatment; and (3) what changes could be found in lipid profiles before IFN treatment and at 6 month after the treatment.
Results: There were no significant relationships of genotype, viral load, ALT, hepatic histology, age, or BMI with lipid profile. The mean levels of pre-treatment total cholesterol (TC) and HDL-C were significantly higher in females (169.3 ± 24.2 mg/dl and 60.6 ± 19.1) than males (149.3 ± 24.5 mg/dl and 48.1 ± 12.2) (both $P<0.05$). No significant difference in the mean levels of pre-treatment triglycerides and LDL-C was found between male and female patients. The mean levels of pre-treatment TC were significantly higher in 22 patients (13 males and 9 females) with sustained HCV clearance (184.3 ± 16.3 mg/dl) than 67 patients (42 males and 25 females) without its clearance (163.9 ± 24.6 mg/dl) after IFN treatment ($P<0.05$), but the other pre-treatment lipid levels were not associated with whether HCV was eliminated by IFN or not. On the other hand, in both sexes, the TC and HDL-C changes after IFN treatment was significantly higher in patients without HCV clearance (+11.4 % and +11.4 % in males; +16.4 % and 10.1 % in females) than those with sustained HCV clearance (+4.0 % and +2.2 % in males; +9.8 % and 6.9 % in females) (all $P<0.05$). The BMI did not significantly change in all patients before and after IFN treatment.
Conclusions: The pre-treatment TC is a predictor of response to IFN treatment in chronic hepatitis C, and IFN can increase TC and HDL-C even in patients with no elimination of HCV.

PERFTORAN® AS AN INTRAVASCULAR SORBENT OF CHOLESTERYL ESTERS

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PERFTORAN® preparation of emulsified perfluorochemicals (PFC) is used nowadays for intravenous infusions for improvement of microcirculation. It comprises the mixture of fluorinated forms of decalin (PFD) and paramethylcyclohexylpiperidine (PMCP). Difference in the hydrophobic properties of the two PFCs produce particles of a certain structure with planar molecules of PFD in the core and PMCPin the peripheral zone. The PFC particle structure may determine the sorbent activity of emulsion while its circulation in the bloodstream which lasts for 24 hs. In in vivo experiments Perftoran was infused into the canicular vein of Wistar female rats of 160-180 g body weight. Animals were killed 1 h, 3 hs, 6 hs and 24 hs after infusion. The PFCs were extracted from blood and their lipid content was analyzed by means of the thin-layer and gas chromatography. The lipid composition reached stabil values up to the 6-th hour of circulation. PFC particles absorbed both polar and nonpolar lipids such as phospholipids (PhL), free cholesterol (Chol), esterified cholesterol (CE), free fatty acids (FFA) and triglycerides (TG). The Phol/Chol ratio was 1:1. Total PhL consisted of phosphatidylcholine (16,6%), phosphatidylethanolamine (12,3%), phosphatidylserine (11,4%) and sphyngomyeline (59,7%). The composition of nonpolar lipids was as follows: FFA- 14,3%, TG-31,5%, CE-54,2%. The CE species of fatty acids were represented mainly by oleic acid (14,7%), a number of 20:n acids (34,9%) and 22:n acids (23,8%). Thus in the bloodstream emulsion particles spontaneously change their surface and core structure according to the chemical properties of PFC. Moreover, they become adaptive to the intravascular environments resembling to some extent lipid proportions of LDL. PERFTORAN may be used as a tool for investigation of lipid transport system, it can find a certain clinical application as an intravascular sorbent of Chol and CE.

THE EFFECTS OF RITONAVIR ADMINISTRATION ON LIPID AND GLUCOSE METABOLISM IN APOE*3 LEIDEN TRANSGENIC MICE

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The introduction of highly active antiretroviral therapy caused a significant decrease in the morbidity and mortality associated with HIV-infection. Currently available drugs however, can cause the lipodystrophy syndrome which is characterised by changes in body fat distribution and several metabolic abnormalities such as hyperlipidaemia and insulin resistance. ApoE*3 Leiden transgenic mice have a humanised lipoprotein profile and are susceptible to diet-induced hypertriglyceridaemia. The aim of this study was to investigate the effects ritonavir (RTV) on lipid and glucose metabolism. In the experiments female apoE3*Leiden mice treated with RTV were used and mice fed only the western type diet were used as appropriate controls.

After administration of RTV for 3 weeks at 35 mg/kg/day, plasma triglycerides (TG) increased from 2.7 to 5.4 mmol/l (P=0.004) and plasma cholesterol increased from 12.7 to 15.3 mmol/l (P=0.017). However, when compared to controls, hepatic VLDL-TG production was not increased after injection of Triton WR 1339. An oral fat load experiment showed a significantly increased postprandial plasma TG response 4 hours after administration of an intra-gastric olive oil bolus. *In vivo* lipolysis of labelled VLDL-like particles showed a significantly decreased uptake of albumin-bound fatty acids in the visceral fat pads of RTV-treated animals when compared to controls. In the subcutaneous fat pads both the uptake of albumin-bound fatty acids and of VLDL-derived fatty acids were decreased in mice that were administered RTV. In the livers of RTV-treated mice an increased triglyceride content was found compared to controls (P=0.014). Hyperinsulinaemic euglycaemic clamps were performed after four weeks of drug administration to investigate whole-body and liver-specific insulin sensitivity. Interestingly, hyperinsulinaemic euglycaemic clamps showed a significantly diminished inhibition of hepatic glucose production in RTV - treated mice by insulin.

The dramatic increase of plasma TG in these mice was not caused by an increase in hepatic VLDL-TG production but could partly be caused by inhibition of the uptake of fatty acids in visceral and subcutaneous fat pads. In addition RTV caused increased hepatic TG content with associated hepatic insulin resistance.

ATAZANAVIR (ATV) IS UNIQUE AMONG (PIs) IN ITS EFFECTS ON LIPIDS. A Lazzarin[1], K Lichtenstein[2], B Cloter[3], T Kelleher[4], M Giordano[4], S Schnittman[4] – [1]S. Raffaele Hospital, Italy; [2]University of Colorado Health Sciences, Denver, USA; [3]IrsiCaixa Foundation, Spain; [4]Bristol-Myers Squibb

As a class, PIs have been associated with lipid elevations that may increase cardiovascular risk. The addition of the PI, ritonavir (r), as a boosting agent to pharmacokinetically enhance primary PI exposure has also been correlated, in some cases, to greater lipid elevations than seen when the primary PI is administered alone. ATV is a potent once-daily PI that has demonstrated comparable clinical efficacy to standard-of-care in treatment-naïve and, when boosted with r, to LPV/r in treatment-experienced patients. ATV, alone (400 mg) or boosted (300 mg) with RTV (100 mg), has been studied with respect to its effects on lipid levels and the need for lipid-lowering therapy as compared to other PIs. Representative trial results are shown.

Clinical Trial	BMS (008) Treatment-naive		BMS (043) Treatment-experienced		BMS (045) Treatment-experienced	
Treatment arm	ATV	NFV	ATV	LPV/r	ATV/r	LPV/r
N, treated	178	91	144	146	119	118
	Mean Percent Change, Baseline to Week 48[†]					
TC	+5	+25*	-2	+12*	-8	+6*
Fasting LDL-C	+5	+23*	-6	+3*	-10	+1
HDL-C	+12	+8	+9	+10	-7	+2
Fasting TG	+7	+50*	+1	+53*	-4	+30*

*-C=cholesterol, LPV=lopinavir, NFV=nelfinavir, TC=total cholesterol, TG=triglycerides *P<0.01, ATV arms vs comparator PIs, †LDL/HDL Week 56 (008)*

In another study, switching from NFV to ATV (AI424-044) resulted in lipid improvements within 12 weeks. The use of lipid-lowering therapy was significantly reduced in ATV and ATV/r-treated patients compared to those treated with LPV/r in studies 043 and 045. These results demonstrate that ATV, whether administered as a single PI or boosted with ritonavir, has favorable effects on lipids in comparison to other PIs.

THE EFFECTS OF EXEMESTANE ADJUVANT THERAPY ON THE LIPID PROFILE AND BODY COMPOSITION IN POSTMENOPAUSAL BREAST CANCER PATIENTS.

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Exemestane, a third-generation aromatase inhibitor, represents a new development in the treatment of estrogen-positive breast cancer; thanks to its irreversible inhibition of the aromatase enzyme causes permanent inactivation. Recently Exemestane therapy after two to three years of Tamoxifen therapy significantly improved disease-free survival as compared with the standard 5-year Tamoxifen treatment. This study aimed to evaluate the effects on lipid profile and body composition of the shifting from tamoxifen to exemestane. Fifty-one postmenopausal women (mean age: 61.0±5.9 yrs) with resected breast cancer, a body mass index (BMI) of 25-35 kg/m^2, and at least two years adjuvant Tamoxifen treatment were randomised to continue Tamoxifen (n = 25; Tam) or switch to Exemestane (n = 26; Exe) without changing their dietary habits. Anthropometric data and lipid profile were obtained one week before randomisation, and then every three months, while DXA (Hologic) body composition were assessed every six months. The two groups were well balanced at baseline. In the Exe group HDL-C resulted significantly lower (-20,7%, $p<0.05$ at 12-moth) and LDL-C significantly ($p<0.01$) higher than basal values (+23.3%, +24.9% e +21.4%, respectively at month 3, 6 and 12). HDL-C/LDL-C ratio significantly decreased starting from 3-month (- 21.0%, $p<0.05$) up to 12-month (-28.3%, $p<0.01$). No changes in lipid profile were observed in Tam group. In the Exe group, but not in the Tam group, fat mass (FM) showed a significant decrease after 12 months, the between-group difference was statistically significant ($p<0.01$). The LM/FM ratio significantly increased in the Exe, but not in the Tam group; the between-group difference was statistically significant ($p<0.05$). These findings indicate that Exemestane has a more favourable effect on body weight and FM than Tamoxifen, whereas Exemestane is unable to maintain positive effects on lipid profile induced by Tamoxifene. Therefore, the choice between Exemestane and Tamoxifen in the first-line treatment of breast cancer should take into account also individual cardiovascular risk.

A NEW MASS SPECTROMETRIC PLATFORM FOR THE ASSESSMENT OF SERUM PLANT STEROLS

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Introduction: Plant sterols (phytosterols) are structurally related to cholesterol. Recent studies suggest that elevated plasma phytosterol concentrations may also be associated with coronary heart disease. However, epidemiological studies to clarify the potential role of phytosterols in coronary artery disease were difficult to perform because of the lack of a simple high throughput analytical method. The aim of our study was to develop and to evaluate a rapid, simple and robust method for the simultaneous quantification of relevant phytosterols in human serum.

Methods: A novel analytic platform based on liquid chromatography and tandem mass spectrometry (LC-MS/MS) using atmospheric pressure photoionization (APPI) was applied. In a simple pre-treatment step only 20 µL human serum were diluted with methanol. After centrifugation, free and esterified ß-sitosterol, campesterol, brassicasterol, and stigmasterol could be simultaneously determined by LC-MS/MS.

Results: The detection limits of the different phytosterols ranged between 0.24 and 0.42 µg/L. Compared with gas chromatography-mass spectrometric method (GC-MS) the analytical sensitivity was 100 to 250 fold higher. The linear ranges were between 1 and 1000 µg/L. The within and between run imprecision ranged between 4.1 and 7.5 %. The total time of sample pretreatment and analysis could be reduced from about three hours (GC-MS) to 15 minutes.

Summary: Our new method allows for the first time a rapid and simultaneous determination of free and esterified phytosterols in serum samples using LC-MS/MS.

A NEW ANALYTICAL PLATFORM FOR CLINICAL PROTEOMICS OF HUMAN SERUM

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Introduction: Protein/peptide biomarkers in serum are potential diagnostic tools for the early detection of chronic inflammatory diseases as atherosclerosis. Common laboratory diagnostic tests detect only a small range of proteins, which are associated with the vascular system (e.g. cell adhesion molecules, acute phase proteins). Mass-spectrometry-driven proteomic analysis is a promising approach for the rapid detection and identification of disease specific biomarkers and proteomic patterns of different body fluids. However, proteom analysis of serum samples requires highly sensitive and specific preanalytical separartion steps. We present the clinical evaluation of a new protein separation procedure, which is now suitable for the detection of clinical proteomic patterns with subsequent MALD-TOF mass spectrometry.
Methods: Peptide and protein separation was performed using a novel technique by magnetic beads with specific surface functionalities (C3, C8, C18). These different beads allow a rapid separation of specific peptide and protein fractions from serum (5 µl). Subsequent proteom profiling was performed using a MALDI-TOF mass spectrometer (Autoflex, Bruker Daltonics).
Results: The protein separation of human serum samples and peptide standards using magnetic beads showed comparable reproducibility. C3-beads were more capable to retain peptides of molecular weights >3000 Da than C8 and C18 beads. No change was observed in frozen samples (-80°C). Repeated freezing and thawing and postprandial hyperlipemia did also not influence the proteomic pattern in the small mass range using C3, C8 and C18 beads (<20 000). However, the proteomic pattern of serum did change with the time after blood sampling (before centrifugation) and at room temperature.
Summary: The magnetic bead based chromatography system combined with MALDI-TOF technology enables an excellent biomarker pattern profiling from biological samples. This first evaluation of the preanalytical conditions of protein separation in serum samples allows now the use of the proteom analytical platform for clinical studies in patients with coronary heart disease and experimental animal models of atherosclerosis. The identification of proteomic patterns in serum specific for atherosclerosis will open new diagnostic und therapeutic targets for the prevention of the disease.

HIGH LEVELS OF LP(a) IN PATIENTS WITH END-STAGE RENAL DISEASE (ESRD), ARE CLOSELY RELATED TO HIGH LEVELS OF C REACTIVE PROTEIN (CRP).

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The mortality in patients with end-stage renal disease (ESRD) is consistently elevated. Cardiovascular disease is the major cause of mortality, accounting for 50% of all deaths in this population. Several studies reported high levels of atherogenic Lp(a) lipoprotein in these patients. C-reactive protein (CRP), the prototypical acute phase protein in humans, seems to increase its plasma levels to the general population when there is a vascular inflammation. Therefore, we studied the role of CRP as predictor for cardiovascular diseases, as far as an inflammatory condition seems to participate in the pathogenesis of atherosclerosis.
The purpose of this study is to evaluate the serum lipids ($ApoA_1$, ApoB, Lp(a) in patients on chronic hemodialysis in relation to CRP plasma levels. The following parameters were determined in 75 stable hemodialysis patients: CRP (nephelometric assay) – Lp(a) (turbidimetric end point method) – $ApoA_1$, ApoB (immuno-turbidimetric test) – cholesterol, triglycerides, HDL (enzymatic methods).
CRP was found to be elevated (CRP>0,8 mg/dl) in 37 patients (group A 2,97±5,07), and low to the rest 38 patients (group B 0,38±0,21). In 72,3% of group A patients, high levels of Lp(a) were determined (Lp(a) > 300mg/lt). The same high levels of Lp(a) were measured in only 27,1% of group B patients (541±425 vs 350±317, p=0,023 - Man Whitney Test).
Additionally we found a significant positive correlation between CRP and Lp(a) only in group A. (r=0,385, p=0,019). The concentrations of $ApoA_1$ and ApoB had no significant difference between the two groups A & B (114±32 vs 123±25, p=0,160 for $ApoA_1$ and 99±29 vs 98±31, p=0,190 for ApoB).
No difference was also found between the two groups A & B, as far as it concerns cholesterol, triglycerides and HDL. On the contrary, the previous reported parameters, had significant differences, compared to the same parameters which we measured in 45 controls.
CONCLUSION: These results suggest that a considerable number of hemodialysis patients exhibit an activated acute phase response, which is closely related to high levels of atherogenic vascular risk factors such as Lp(a).

RELATIONSHIP BETWEEN SERUM CRP, ARTERIAL INTIMAL HYPERTROPHY AND PLAQUE CRP and WIDESPREAD VASCULAR CRP PRODUCTION.

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Raised circulating C-reactive protein (CRP) predicts vascular events. Produced predominantly by liver, CRP may also be derived from fat, including perivascular fat and vasculature. A relationship between circulating CRP levels, intimal hypertrophy and plaque CRP and of local CRP production would support a direct role for CRP in atherosclerosis. Vascular tissue from subjects undergoing coronary artery bypass grafting surgery (CABGS) (n=28) and carotid endarterectomy (CEA) (n=25) were studied. Arterial intima-media ratio (IMR) and CRP were determined by immunohistochemistry. CRP mRNA was assessed by real-time polymerase chain reaction. In a separate series of subjects undergoing CABGS or angiography (n=54) CRP was measured in aortic and coronary sinus blood. Serum hsCRP correlated with IMR (r=0.64, p=0.001) in non-atherosclerotic arteries independent of age, BMI, blood pressure and diabetes, and with plaque CRP staining (r=0.57, p=0.009). CRP mRNA was present in all plaques, non-atherosclerotic artery and atrium, but not in saphenous veins. No difference was seen in CRP mRNA expression between plaque and non-atherosclerotic tissue, although CRP protein was not present in non-atherosclerotic tissue. There was a CRP gradient across the coronary circulation (mean aortic CRP 4.3±0.8 mg/l vs coronary sinus 5.8±1.2 mg/L, p<0.05). In conclusion, serum hsCRP correlates with intimal hypertrophy and with plaque CRP. Vascular CRP mRNA expression and a coronary CRP gradient suggest vascular secretion. Whilst CRP may be widely produced presence of CRP protein only in plaque may represent CRP retention via binding to modified lipoprotein. CRP production and arterial retention may represent therapeutic targets.

PATIENTS WITH PERIPHERAL VASCULAR DISEASE ELICIT ELEVATED UPPER-LIMB PULSE WAVE VELOCITY AT REST AND DURING ACUTE EXERCISE STRESS

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Introduction: Peripheral vascular disease (PVD) is one of the major manifestations of atherosclerosis. Stiffening or arteriosclerosis of the arterial wall can be assessed non-invasively by measurement of pulse wave velocity (PWV). Little is known about the PWV of the upper-limb in patients with PVD, therefore, we examined carotid-radial PWV at rest and during a single bout of isometric handgrip exercise (ISOMEX) in PVD patients and compared them to healthy subjects.
Methods: Dominant-arm PWV and non-invasive haemodynamics (BP & HR) were examined in 10 PVD patients (age (mean ± SD) 63.8 ± 9.4, BMI 25.1 ± 2.4 kg/m^2) versus 10 healthy controls (age 62.3 ± 11.2, BMI 27 ± 3.7 kg/m^2) at rest, during and into recovery from 3-minutes of supine sub-maximal ISOMEX (30 % MVC) of the non-dominant arm.
Results: PVD patients had significantly higher resting PWV (9.52 ± 1 m/sec) compared to controls (8.37 ± 0.8 m/sec) ($P \leq 0.01$). ISOMEX PWV was significantly greater in PVD patients (10.88 ± 1.2 Vs 9.17 ± 0.9 m/sec, $P \leq 0.002$), as was the PWV measured post-exercise (9.82 ± 1.5 Vs 8.62 ± 1.1 m/sec, $P \leq 0.03$). The actual PWV increase induced by ISOMEX was also larger in the PVD patients ($P \leq 0.05$).
Conclusions:
Vascular stiffness of the upper limb is increased in PVD patients. The ISOMEX PWV stress test elicits marked changes of PWV in this disorder, as a result of amplified efferent sympathetic nervous outflow. Such a test may be valuable for further characterising PVD patients and evaluating the effects of therapy on sclerotic alterations of the arterial wall.

THE RELATIONSHIP OF PHYTOSTEROLS TO INCIDENT CHD IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) COHORT

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Phytosterols (plant sterols) have been associated with premature atherosclerosis in individuals with the rare genetic disorder sitosterolemia, and several observational studies have shown modest but significant elevations in phytosterol concentrations (or their ratios to cholesterol) among coronary heart disease (CHD) patients without sitosterolemia. A recent nested case–control study within the Prospective Cardiovascular Münster (PROCAM) cohort demonstrated that elevated concentrations of sitosterol were associated with a 3-fold increased risk of subsequent coronary events in men at high global risk for CHD.

The purpose of this study is to determine whether plasma phytosterol concentrations (sitosterol and campesterol), measured in individuals who are free of clinical CHD at baseline, are associated with development of incident CHD events independent of established major CHD risk factors in men and women in the ARIC cohort. The ARIC study is a prospective study to investigate the etiology of atherosclerosis in a bi-racial cohort of 15,792 adults from 4 U.S. communities who participated in 4 study visits and annual questionnaires from 1987 to 1998.

A case–cohort study design will be used to compare 775 incident CHD cases with a stratified random sample of 936 subjects from the cohort. Cox proportional hazards survival models (adjusted for key demographic factors) will be used to assess the association between phytosterol concentrations and CHD in individuals with and without established major CHD risk factors. Results will also be reported for various subgroups.

OXIDIZED LDL INDUCE THE PRODUCTION OF TNF-ALPHA BY HUMAN THP-1 CELLS IN CONTRAST TO OXIDIZED ALBUMIN

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It is now well accepted that oxidative modification of low-density lipoprotein (LDL) contributes to the pathology of atherosclerosis. Myeolperoxidase (MPO), an enzyme secreted by activated phagocytes is considered to be responsible for LDL oxidation, in the intima, converting the LDL into a high-uptake form for macrophages. In this study we aim to show that MPO-oxidized-LDL are specifically involved in the initiation of an ongoing inflammatory response that is mediating all stages of atherosclerosis. To do so, LDL and albumin were oxidized by recombinant MPO, checked out on agarose gel and using anti-Mox-LDL monoclonal antibodies, Mox-albumin was not recognized showing the specificity of these antibodies for Mox-LDL. The production of TNF-α in the supernatant was measured after 4 h of incubation of Human THP-1 cells in the presence or absence of 100µg/ml of either Mox-albumin or Mox-LDL. Mox-LDL (2 ± 0.2) induced a 2-fold increase in the TNF- α production compared to native LDL (1.2 ± 0.28) and cells alone. On the other hand, native albumin (0.6 ± 0.1) decreased this production while the Mox-albumin (1 ± 0.23) does not produce TNF-α. As a positive control we used LPS (4.9 ± 0.19) known to produce a high amount of TNF-α. In conclusion, we may propose that the oxidized LDL in the circulation in opposition to native LDL, are able to trigger an inflammatory process that is known to promote initiation and evolution of atherosclerosis while albumin has no apparent role in this initiation.

MILDLY OXIDIZED LOW DENSITY LIPOPROTEINS INHIBIT THE *IN VITRO* INDUCTION OF THE SPECIFIC ANTIBODY RESPONSE TO *CANDIDA ALBICANS* BY AN EARLY UP-REGULATION OF IL-1 β PRODUCTION

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Several lines of evidence support the view that oxidative modification of LDL plays a central role in the development of atherosclerosis. Oxidized LDL exert various biological effects, both on vascular wall cells and on cells infiltrating the lesions. It has been demonstrated that early atherosclerotic lesions in humans and mice contain significant numbers of T cells and B cells. The present study was designed to evaluate the effect of mildly oxidized LDL (mLDL) on the induction and regulation of the specific antibody response *in vitro*. Human peripheral blood mononuclear cells from healthy donors were induced to mount a specific antibody response to the *Candida albicans* antigen. We found that mLDL significantly inhibited the induction of the anti-*Candida albicans* antibody response. Lipid extracts from mLDL were able to reproduce the same effects as the lipoprotein. The down-regulating effect on the specific antibody production induced by mLDL was abrogated by blocking the major receptors that bind and internalize modified LDL. In the mLDL-treated cultures an early increase of IL-1β production was observed and the addition of anti-IL-1β antibody abrogated the mLDL-induced inhibitory effect. Moreover, the addition of IL-1β to the cultures inhibited the induction of the specific antibody response, as well as mLDL. On the hand, mLDL up-regulated PWM-induced polyclonal Ig production. In the same cultures antibody to mLDL (IgM isotype) were found. Our results could add new insight to the key role played by mLDL as modulators of B cell function contributing to understand the mechanism by which a specific component of the immune system impacts atherogenesis .

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THIOL-CONTAINING MOLECULES DIRECTLY INTERACT WITH MYELOPEROXIDASE TO INHIBIT LDL OXIDATION BY THE MYELOPEROXIDASE / H_2O_2 / Cl^- SYSTEM.

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The myeloperoxidase (MPO) / H_2O_2 / Cl^- system is involved in LDL oxidation, a pathogenic factor in the development of atheromatic lesions. The phenomenon is preceded by a coupling between MPO and LDL where apoprotein-B100 is modified. The present study documents the effects of thiol-containing molecules such as glutathione (GLU), captopril (CAP) and N-acetylcysteine (NAC) and its lysinate salt (NAL) on both the MPO system and LDL oxidation.

The quantity of methionine required to inhibit 50 % of oxidation of thiols by the MPO system was first determined. Then, the inhibition of LDL oxidation in relation to several concentrations of the different investigated molecules (5 to 300 µM) was assessed by an ELISA system. Finally, the interval of time required to scavenge the "compound II", an oxidised form of MPO resulting from the reaction with H_2O_2, was measured.

The results show that thiol-containing molecules, which have well known antioxidant properties against oxygen-derived species including HOCl, are also good scavengers in the MPO system (GLU > NAC = NAL > CAP, $P<0.01$). Furthermore, they inhibit LDL oxidation in spite of the coupling between MPO and LDL (NAC = NAL ~ CAP > GLU, $P<0.01$). This inhibition is a consequence of a direct interaction with oxidized MPO (compound II) where NAC and NAL are more efficient than CAP and GLU ($P<0.01$). The size and the shape of the molecule could be crucial to modulate the extent of this effect.

OXIDATION OF LOW-DENSITY LIPOPROTEINS BY MYELOPEROXYDASE AT THE SURFACE OF ENDOTHELIAL CELLS: AN ADDITIONAL MECHANISM TO SUBENDOTHELIUM OXIDATION.

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According to the oxidation hypothesis, low-density lipoproteins (LDL) are trapped and retained in the intima where they undergo oxidative modification. MPO has been implicated as an enzymatic catalyst of LDL oxidation, in the intima, converting the LDL into a high-uptake form for macrophages. We hypothesize and try to show that the oxidation of LDL might also occur at the surface of the endothelial cells under physiological conditions. To do so, Ea.hy926 endothelial cells were incubated for 24h in medium containing native LDL at concentrations ranging from 500 to 2000µg/ml, in the presence or absence of 2.5 µg/ml of recombinant-MPO. The production of MPO-oxidized LDL (Mox-LDL) was measured by an ELISA assay. The incubation of endothelial cells with native LDL in conjunction with MPO resulted in a 1.5 to 2.5-fold stepwise increase in Mox-LDL production (ANOVA, $p<0.001$), while at a constant concentration of native LDL (1500 µg/ml), MPO significantly increased the production of Mox-LDL (2 to 2.6-fold increase in comparison with the controls, $p<0.001$) in a dose-response effect of MPO concentration ranging from 0 to 3µg/ml. In this study we tried to show that a cellular mechanism of LDL oxidation by myeloperoxidase already occurs at the surface of the endothelial cells and may not be restricted to the sub-endothelial space.

ANTIBODIES AGAINST OXLDL IN ACUTE CORONARY SYNDROMES PATIENTS.

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Oxidative stress and inflamation play a significant role in atherogenesis. Oxidized low-density lipoprotein (OxLDL) present at sites of atheromatous lesion are cytotoxic and induce various cellular responses such as vasoconstriction, expression of adhesion molecules and cellular proliferation. OxLDL are antigenic, resulting generation of autoantibodies that may reflect the extent of in vivo oxidation of LDL. The aim of our study was to evaluate the plasma level of autoantibodies against oxLDL in acute coronary syndromes' patients. We have studied 40 patients with acute coronary syndromes and 34 healthy controls. Antibodies against OxLDL were measured by ELISA kit (Biomedica). Plasma concentration of anti-oxLDL Ab were significantly higher in coronary syndromes patients (542 IU/l) than in the control group (292 IU/l) $p<0,05$. Our study suggests that autoantibodies against OxLDL are proatherogenic and may be a useful marker to predict acute coronary syndromes but it requires further investigation.

COMPARISON ANTICARDIOLIPIN AND ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS THAT RESPOND AND NOT RESPOND TO STREPTOKINASE

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Background: Myocardial infarction is one of the most causes of hospitalization, and its treatment is mainly reperfusion of infarcted myocardium. Streptokinase therapy is a medical approach for reperfusion therapy but response to it is variable between patients which may be due to antistreptokinase antibodies or other antibodies such as antiphospholipid or anticardiolipin antibody. Therefore this study will compare the blood levels of anticardiolipin and antiphospholipid antibodies and patients that respond and not respond to streptokinase.
Methods: Each group contains 26 male patients with myocardial infarction who aged 40-60 years and experienced the first time MI. They didn't have the history of autoimmune disease, pulmonary thromboembolism, Arterial thromboembolism, stroke and deep vein thrombosis. Their response to streptokinase were measured according to their first ECG and the ECG of 90-180 minutes after streptokinase. Reduction of more than half of ST elevation was considered as their response to the treatment. The levels of anticardiolipin and antiphospholipid antibodies were measured by ELISA. Relation between the mean level of these antibodies and their response to streptokinase was assessed.
Result: The Mean level of IgG Anticardiolipin in patients that response to streptokinase was significantly lower than the other group ($P=0.009$) but the mean level of other antibodies: IgM Anticardiolipid($p=0.554$), IgG Antiphospholipid($p=0.251$) and IgM Antiphospholipid($p=0.301$) was not significantly different ($P=0.554$, $P=0.251$, $P=0.301$).
Conclusion: Based on the results, can say that anticardiolipin antibody has an effect on the response to streptokinase. This antibody can inhibit the fibrinolytic effect of plasminogen and can neutralize the normal dose of streptokinase.

FIBRINOLYTIC ACTIVITY OF *AMIRKABIRIA ODORATISSIMA*

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Background: Fibrinolytic agents like streptokinase (SK) and urokinase (UK) are urgent treatment of thromboembolism. Besides thrombolysis, these medications have side effect like: generalized lytic state, serum fibrinogen decrease, effect on body hemostasis and life threatening bleedings, allergic reaction and urticaria.
Studies have shown that some of the patients don't response to SK, for example who have used SK one or two times or patients with streptococcal disease.
In this study, the fibrinolytic activity of *Amirkabiria odoratissima mozaffarian* to find the natural compounds that can be used in treatment of thromboembolism is studied.
Methods: Polyphenolic extract and the essential oil were prepared. Then essential oil was analyzed by G C. Mass. Fibrinolytic effects of SK (as positive control), extract and essential oil was studied by flourimetric method.
At first fibrinogen was labelled with FITC (Flourescein Isothiocyanate) and then labelled thrombus was produced in plasma with calcium ion.
Then SK (100-1000 unit/µl) and essential oil at dilutions of (1/10, 1/100, 1/1000) V/V and extract (1/1, 1/10, 1/100, 1/1000) V/V was added and after 15, 30 and 60 minutes, fluorescence was measured.
Result: Results showed linear relationship between fluorescence and SK concentration between 300-700 unit/µl. Essential oil was more effective in thrombolysis than the extract of and exposure of both of them to environment has shown a little increase with passing of time. The extract had relative effect on thrombolysis.
Conclusion: Results has shown that *Amirkabiria odoratissima mozaffarian* has considerable fibrinolytic effects. So, study of different fractions of this plant and isolation and purification of them for fibrinolytic effects is recommended.

MONOCLONAL ANTIBODIES RECOGNIZING ATHEROMATOUS AORTA

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To study how cholesterol accumulate in atheroma, novel monoclonal antibodies were prepared, using crude homogenate of atheroma as immunogens

EMR1a/212D antibody recognizing extra cellar matrix with lipid-laden deposits was selected by histochemial staining. The antigen was deduced vitronectin from cDNA library.

FOH1a/DLH3 antibody recognizing oxLDL, epitope of which was 5- and 9-phosphatidylcholine that can form complexes with polypeptides including apoB. Significant correlations between oxLDL and CHD patients was observed from clinical study.

ASH1a/256C antibody recognizing atherosclerotic lesions in human aortae was selected. Epitope must be PC-neutral lipid complex which may involve in foam cell apoptosis. Atherogenesis will be discussed from these antibodies

PATIENTS WITH CROHN'S DISEASE AND THE CAUSAL RELATIONSHIPS BETWEEN INFLAMMATION, HDL LEVELS AND ATHEROSCLEROSIS PROGRESSION

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Introduction HDL is best known for its anti-atherogenic properties: individuals with decreased HDL levels have increased cardiovascular disease risk. Decreased HDL is also observed in shock patients and in patients with inflammatory bowl diseases (IBD). To draw attention to its multifuctionality, HDL in patients in the active and remissive stages of Crohn's disease were investigated. **Methods** Fifteen controls and 21 Crohn's patients, 11 in remission and 10 with active disease, participated. Lipid profile, serum CRP and clinical status according to the Harvey-Bradshaw Index (HBI) were obtained. 'Active' disease was defined as an HBI >4 *and* a CRP 10mg/L. **Results** CRP in controls ((0.8(SD0.4)mg/L) was decreased if compared to patients in remission (2.6(2.7)mg/L) and in active patients (108.8 (113.8)mg/L): p=0.017 and 0.001, respectively. CRP in active patients was increased in comparison to patients in remission (p=0.006). LDL levels in controls and in remissive and active patients were similar (2.64(0.66), 2.54(0.70) and 2.59(0.97)mmol/L, respectively: all p=n.s). HDL in controls and in patients in remission were identical ((1.45(SD0.48) and 1.40(0.46)mmol/L: p=0.797); HDL of patients with active disease was decreased if compared to controls *and* to those in remission: p-values 0.022 and 0.043. In a linear regression analysis ^{e}logCRP and HDL were correlated: r2=0.24, p=0.002. **Discussion** HDL is affected in Crohn's patients in the active but not in the remission phase of the disease. Protracted active disease in Crohn's patients reduces HDL levels, most likely due to inflammatory respons. Inflammation and low HDL are known to increase cardiovascular disease risk. The risk and need for prevention are identified in further studies with validated biomarkers such as carotid ultrasound arterial wall measurements and brachial flow mediated dilatation. Since inflammatory state is documented, patients with Crohn's disease may serve as a model to understand causal relationships between inflammation and atherosclerosis.

A LOW DOSE OF ORAL FAT LOAD IMPAIRS ENDOTHELIAL FUNCTION IN HEALTHY MALES

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Introduction Elevated plasma triglyceride-rich lipoproteins and lipid oxidation products impair arterial endothelial function, accelerate atherosclerosis and increase cardiovascular disease risk. Endothelial function, as early indication of atherosclerosis, can be evaluated with ultrasound flow-mediated brachial artery dilatation (FMD) measurements. Effects of standardized oral fat loading on FMD were investigated. **Methods** Thirty healthy males (age 30.8(SD9.3)yrs; TC 4.7(1.0), HDL-C 1.4(0.3) and TG 1.0(0.4)mmol/L; SBP123(9), DBP72(9) mmHg, BMI 23.3(2.6)kg/m^2) participated. Subjects refrained from nutritional supplements (vitamins, minerals) for 2 weeks, alcohol (1 day) and received standardized meals for 3 days prior to FMD assessments. Brachial artery diameter as measured at start of assessment, and FMD were measured prior to and after 50g saturated fat intake. Brachial artery FMD was induced by release of a cuff that had arrested forearm blood flow for 5 minutes. Arterial diameter at start of assessment and maximal diameter were measured. FMD was defined as % dilatation. **Results** Fat loading increased brachial artery diameter (from 4.22(0.57) to 4.33(0.58)mm: p=0.023), and decreased FMD (5.3(3.3) to 3.3(2.8)%: p=0.024. **Conclusions** Brachial ultrasound FMD measurements can identify exposure to oral fat loading in healthy males. The impaired FMD was, at least partially, accounted for by a fat loading related brachial diameter increase. Our studies indicate that a low does oral fat load in combination with a strictly adhered brachial ultrasound FMD protocol can be used as a tool for risk reduction assessment of vasoprotective agents.

THE RELATIONSHIP OF LARGE ARTERY PROPERTIES WITH HOMOCYSTEINE LEVELS IN HEALTHY SUBJECTS

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Objectives: Plasma homocysteine level (Hcy) is known to be strongly associated with the risk of cardiovascular diseases. Whether this association is caused by the effect of Hcy on large arteries, remains unclear.

Design and Methods: Healthy subjects aged 25 – 65 years were randomly selected and examined in the framework of a large population survey. In 290 of them (143 males and 147 females), large artery properties were examined using the Sphygmocor system. Augmentation index (AIx) showing the extent of wave reflection was assessed on the radial pressure wave, central pulse wave velocity (PWV) reflecting aortic stiffness was measured between carotid and femoral artery and peripheral PWV was measured between femoral and dorsalis pedis/tibialis posterior artery. Hcy was assessed using commercial FPIA kits.

Results: Mean values ± SEM are given in the Table:

	Plasma homocysteine quartiles (μmol/l)				P for trend
	<9	<10.7	<13	≥13	(ANOVA)
age [years]	44±1	49±1	50±2	49±2	<0.02
Systolic BP [mmHg]	120±2	126±2	128±3	129±2	<0.02
Diastolic BP [mmHg]	80±1	80±1	80±1	82±1	0.60
Central PWV [m/s]	6.8±0.2	7.2±0.2	7.5±0.3	8.7±0.4	<0.001
Peripheral PWV [m/s]	11.2±0.4	14.0±1.2	14.5±1.6	13.4±1.2	0.96
Peripheral AIx [%]	67.4±2.3	72.4±2.4	75.2±2.8	69.1±3.2	0.40

Conclusions: Aortic stiffness, but neither stiffness of peripheral large arteries, nor wave reflection, is associated with plasma Hcy levels independently of conventional cardiovascular risk factors.

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INTERLEUKIN-1 RECEPTOR ANTAGONIST ATTENUATES FATTY STREAK FORMATION IN LDL RECEPTOR KNOCKOUT MICE

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IL-1 causes a wide range of inflammatory processes within the atheroma, including those which initiate its development and those which effect plaque stability. The aim of the current study was to examine whether treatment with recombinant IL-1 Receptor antagonist (IL-1Ra) has a beneficial effect on atherosclerosis progression in LDL receptor knockout (LDLR-/-) mice. LDLR-/- female mice (n=28) were fed a high-fat, high-cholesterol Western diet for 4 weeks. Recombinant IL-1Ra (100 μg/mouse) (n=14) was injected i.p every other day, in a total volume of 250μl PBS. Control group (n=14) was treated with PBS alone. The IL-1Ra-treated mice showed a significant reduction in the aortic sinus lesion area, as compared to the control group, 23,732±3,078 *vs.* 34,690±3,053 μm^2 for IL-1Ra and control groups, respectively, ($p< 0.018$). Although IL-1Ra treatment lowered plasma cholesterol levels significantly (602+33 mg/dl compared to 717+35 mg/dl in control group), we suggest that the anti-inflammatory properties of IL-1Ra may play an important role in its anti-atherogenic effects.

SERUM MARKERS OF INFLAMMATION IN APO $E^{-/-}$-MICE

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Both inflammation and dyslipidemia play a role in the pathophysiology of atherosclerosis. ApoE knock-out ($ApoE^{-/-}$)-mice are a well-known model of atherosclerosis and develop aortic plaques on Western type diet within 3-4 months while progression to atherosclerosis is very slow on regular diet. We used this animal model to characterize serum markers of inflammation. The chemokines KC (the mouse analogue of interleukin-8) and JE (the mouse analogue of monocyte chemoattractant protein1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were determined by commercially available ELISA kits (R&D Systems Europe, Abingdon, UK) after eight weeks of Western type diet or regular diet, respectively.
Serum KC and JE are 2-3 fold higher on Western type diet than on regular diet while sVCAM is only slightly increased. The MTP (microsomal triglyceride transfer protein) inhibitor implitapide diminishes the increase in plasma cholesterol induced by Western type diet and also prevents the increase in KC and JE. This result shows that the increase of chemokines in Apo $E^{-/-}$ - mice on Western type diet is closely linked to dyslipidemia.
As a prototype of non-lipid lowering drugs with good efficacy in the ApoE model the ACE (angiotensin converting enzyme) inhibitor captopril was used. Treatment with captopril (280 mg/l drinking water) significantly reduces KC and JE serum levels on Western type diet. This result is probably due to inhibition of the pro-inflammatory effects of angiotensin II.

LOW LEVEL CRP INCREASES ADHESION OF LEUKOCYTES TO ENDOTHELIAL CELLS AND DECREASES PHAGOCYTE RESPIRATORY BURST ACTIVITIES

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Low systemic levels of C-reactive protein (CRP) between 1 and 10 mg/L have been shown to be highly predictive for future atherosclerosis-mediated cardiovascular events. While the value of low level CRP as an excellent biomarker of chronic inflammation has been established in several studies its possible role as a mediator actively partaking in atherosclerotic lesion formation remains unclear.
In our *in vitro* study we investigated the effects of low concentrations of CRP on the adhesion of polymorphonuclear leukocytes (PMN) and murine macrophages (RAW cell line 264.7) to human umbilical venous endothelial cells (HUVEC) and on the generation of reactive oxygen (ROS) species by PMN.
Co-incubation of HUVEC with PMN and human recombinant CRP (Calbiochem, 1.5, 3.0, and 5.0 mg/L, respectively) for 1 h resulted in a dose-dependent increment of leukocyte adhesion to HUVEC (147±25%, 179±14%, and 207±20%, respectively). Furthermore, CRP in the indicated concentrations enhanced the native LDL- and oxidized LDL-induced adhesion of PMN substantially. Similar results were obtained using macrophages instead of PMN.
Addition of CRP (1.5-5.0 mg/L) to activated PMN was followed by a dose-dependent reduction of ROS generation. CRP at 3.0 mg/L diminished FMLP-induced chemiluminescence (CL) by 30%, zymosan-induced CL by 90%, and oxidized LDL-induced CL by 15%.
In conclusion, the present results show that CRP in concentrations predictive for future cardiovascular disease is able to substantially modulate important proinflammatory processes.

INTERRELATION OF LIPIDES, INTERLEUKIN-1, INTERLEUKIN-6 AND C-REAKTIVE PROTEIN IN PATIENTS WITH CAROTID ASYMPTOMATIC STENOSIS

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For establishing relation between Atheroscerosis and inflammation multicomponent mechanisms it is very important at present to study interleukins(IL) and C-reactive protein (CRP) together with blood lipides.
The aim of the work is to establish correlation between interleukins 1 and 6 (IL-1, IL-6), CRP and different classes of lipides in patients with Carotid Asymptomatic Stenosis (CAS)
69 patients were examined (age within 33-63, 47-male, 22-female), who had different carotid artery lesions without clinical signs. The control group consisted of 25 practically healthy from 32 to 52 years old persons. According to carotid stenosis the patients were divided into 2 groups: I - hemodynamically insignificant – stenosis degree<50% (37 patients); II - hemodynamically significant – stenosis degree>50% (32 patients);
All the patients underwent carotid extracranial duplex scanning, laboratory examinations and the above parameters study.
Total cholesterol (TCh) amount in both groups was increased and varied between 5.5mol/l – 7.4mmol/l
In the group II, along with HDL Cholestrol decrease (up to 1.1 mmol/l), LDL Colesterol increased in number up to 4.7 mmol/l. The same changes were noted between Apo-A_1 and Apo-B.
The IL-1 and IL-6 concentration was observed to show the tendency of growth in 60% of the second group patients (19 patients), in which the number of CRP was increased (7.0±0.4 mg/dl).
LDL Cholesterol , Apo-B, CRP, IL-1, IL-6 and TCh parameters were in positive correlation with the degree of CAS.
Negative correlation was noted between HDL Cholesterol, Apo-A_1 and CAS degree.
Thus, in asymptomatic carotid atherosclerotic lesions the character of the laboratory parameter changes, studied by us, should point at carotid atherosclerois and inflammation entire pathogenesis. Therefore, we think it possible to use CRP, IL-1 and IL-6, together with blood lipids, as laboratory markers for diagnosis and treatment of carotid atherosclerosis.

NO EFFECT OF C-REACTIVE PROTEIN ON EARLY ATHEROSCLEROSIS DEVELOPMENT IN APOE*3-Leiden/hCRP TRANSGENIC MICE

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Introduction The inflammatory marker C-reactive protein (CRP) is a strong predictor of cardiovascular events in healthy persons as well as in patients with cardiovascular disease. However, it is not clear whether CRP is causally involved in the development of cardiovascular disease. CRP is not a sensitive inflammatory marker in mice, and therefore transgenic mice carrying the human CRP (hCRP) gene, including its flanking regulatory sequences, offer a unique model to study the role of CRP. The purpose of this study was to investigate the hypothesis that CRP is causally involved in the development of early atherosclerosis in apolipoprotein E*3-Leiden (E3L)/hCRP transgenic mice.
Methods The effect of CRP on the development of early atherosclerosis was studied in E3L/hCRP mice (14 males and 13 females); as a reference group 14 male and 13 female E3L mice, a well-established mouse model for hyperlipidemia and atherosclerosis, were used. The mice were fed a semi-synthetic cholesterol-containing diet resulting in mildly elevated plasma cholesterol levels of about 15 mmol/L. After 6 – 7 months the mice were sacrificed and atherosclerosis development in the aortic root area was assessed.
Results Cholesterol exposure did not differ significantly between E3L/hCRP and E3L (312 ± 31 and 325 ± 25 mmol/l*weeks for female mice; 552 ± 61 and 499 ± 96 mmol/l*weeks for male mice, respectively). Plasma CRP levels were 11.6 mg/l in male E3L/hCRP mice, 0.2 mg/l in female E3L/hCRP mice and undetectable in male and female E3L mice. The lesion type and the atherosclerotic lesion area were similar in E3L/hCRP and E3L mice (35250 ± 31285 μm^2 and 36255 ± 27482 μm^2 in female E3L/hCRP and E3L mice, 26402 ± 16973 μm^2 and 24776 ± 20442 μm^2 in male E3L/hCRP and E3L mice, respectively). In female mice all types of lesions were found. In male mice the lesions were mostly type I-III. However, no differences were observed in lesion types between the E3L/CRP and E3L mice.
Conclusion Lesion area and type in E3L/hCRP mice are comparable to those in E3L mice. Therefore, mildly elevated levels of CRP do not contribute to development of early atherosclerosis in mildly hypercholesterolemic mice.

α-TRIFLUOROMETHYL-α-AMINO-β-SULPHONE HYDROXAMATE INHIBITS MMP-2, -3, AND –9 ACTIVITY

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Excessive breakdown of extracellular matrix (ECM) by metalloproteinases (MMPs) occurs in many pathological conditions, such as cancer and atherosclerosis, and thus inhibition of MMPs activity might have therapeutic potential. We have synthesized a new compound named MS560 (α-trifluoromethyl-α-amino-β-sulphone hydroxamate). The introduction of the trifluoromethyl group was expected to be an effective strategy for changing and tuning the binding properties of the hydroxamate to MMPs' catalytic site. We then tested MS560 capacity to interfere with MMPs' expression and activity. The effect on MMP-2 and MMP-9 secretion and gelatinolytic activity was evaluated by gelatin gel zymography using cell-conditioned media by smooth muscle cells (SMCs) and macrophages, respectively. The activity on MMP-3 was measured using the purified enzyme and a fluorescent assay. The incubation of macrophages or SMCs with increasing concentrations of the compound (from 0.05 up to 25 μM) did not affect cellular viability. In addition, the compound affected the secretion of MMP-2 and MMP-9 only slightly in macrophages while it did not have any effect on MMP-2 in SMCs. However, our data showed that MS560 is able to inhibit the gelatinolytic bands at 92 and 72 kDa, corresponding to pro-MMP-2 and pro-MMP-9, (up to 90% and 92%, respectively, $p<0.01$) released into the conditioned medium (IC_{50}: MMP-2 970 nM; and MMP-9 87 nM). MS560 was more active in inhibiting MMP-3 activity (IC_{50} MMP-3 14 nM). Therefore, these results show that MS560 inhibits specifically MMPs' activity at nanomolar concentration highlighting the potential beneficial effect of this type of compounds in the therapeutic control of excessive ECM breakdown.

MATRIX METALLOPROTEINASE 2 AND 9 VALUES IN VARIOUS GROUPS WITH OR WITHOUT DIABETES

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Matrix metalloproteinases (MMPs) are critical for vascular remodelling by regulating degradation of the extracellular matrix. We evaluated MMP2 and MMP9 levels in 4 groups: healthy subjects (1), diabetic patients (2), nondiabetic patients with acute coronary syndrome (ACS) (3), and diabetic patients with ACS (4). We detected by ELISA serum MMP2 and MMP9 levels in 21 healthy subjects aged (mean±SD) 48±9 years, in 20 type 2 diabetic patients aged 53±10 years, in 27 nondiabetic patients with ACS aged 67±11 years, and in 13 type 2 diabetic patients with ACS aged 76±5 years. MMP2 levels were significantly higher in group 3 and 4 ($p< 0.0001$, respectively) compared to groups 1 and 2, while group 3 did not show any significant difference compared to group 4. MMP9 were significantly higher in group 3 and 4 ($p< 0.0001$, respectively) compared to groups 1 and 2. MMP9 were significantly higher in group 4 ($p< 0.042$) compared to group 3.
We can conclude that patients with ACS have MMP2 and MMP9 levels higher than groups 1 and 2, but only group 4 has higher level of MMP9 compared to group 3.

ROLE OF SERUM OSTEOPROTEGERIN IN CAROTID ATHEROSCLER - OSIS AND OSTEOPOROSIS

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Background:Vascular calcification is a significant marker of atherosclerotic disease. Osteoporosis and atherosclerosis often coexist. Osteoprotogerin (OPG) is a soluble protein expressed on the surface of osteoblast-like cells and inhibits the differentiation of osteoclasts. Osteoporosis in post-menopausal women is associated with arterial mineralization. Mice deficient in OPG develop osteoporosis and vascular calcification. The purpose of this study was to determine if OPG is associated with carotid artery calcification and osteoporosis in humans.. **Methods**: Fifteen patients (8 men, 7 women; age=74.9±7.8yrs) who had undergone unilateral carotid endarterectomy (CEA) within the last 18 months were enrolled in the study. The CEA specimens were analyzed by EBCT for their calcium content in terms of Agatson and Volume scores. Their fasting blood samples were analyzed for levels of plasma osteoprotegerin (OPG), serum calcium, and plasma lipids. Their radial bone densities were measured at the wrist in terms of T-Scores and Z-Scores (Sunlight Omnisense 7000S bone sonometer). **Results**: Plasma OPG levels ranged from 1103.8 pg/mL to 3049.2 pg/mL (1934 ± 576) and were significantly higher in patients with lower bone density (r=-0.827, -0.697). Highly calcified CEA tissues were rich in osteopontin (OPN) and poor in OPG, whereas lowly calcified tissues were poor in OPN and rich in OPG. CEA calcium volume was positively associated with age (r=0.633), but did not have a significant correlation with bone density. Lipid levels were not significantly associated with other variables, probably because patients were on statin therapy. **Conclusion:** The elevated concentrations of OPG in serum of subjects with lower bone density, and the abundance of OPG in CEA tissues with low calcification suggest that OPG may play an important role in the mineralization of bone and the calcification of arterial wall. (HL63090).

CYSTSTIN C DEFICIENCY INCREASES ELASTIC LAMINA DEGRADATION AND AORTIC DILATATION IN APO E KNOCKOUT MICE

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The formation of atherosclerotic plaques and arterial aneurysms involves substantial vascular remodeling. We previously showed that compared to normal arteries human atherosclerotic and aneurysmal (AAA) lesions contain more cysteine protease and less of their endogenous inhibitor cystatin C, suggesting an imbalance of proteases/protease inhibitor that would favor vascular wall proteolysis. Thus, impaired expression of cystatin C may aggravate this imbalance and accentuate atherogenesis. To test this hypothesis directly in vivo, we generated cystatin C and apolipoprotein E (Cyst $C^{-/-}ApoE^{-/-}$) doubly-deficient mice fed an atherogenic diet for 12 weeks. Extracts of Cyst $C^{-/-}ApoE^{-/-}$ aortic lesions had augmented activity of the elastolytic enzyms cathepsins S and L and degraded insoluble elastin more rapidly than those from control $ApoE^{-/-}$ mice. Moreover, aortic smooth muscle cells (SMC) isolated from cystatin C-deficient mice showed increased elastolytic and collagenolytic activities after stimulation by pro-inflammatory cytokines in vitro. Cystatin C-deficiency yielded significantly increased elastic lamina fragmentation in the tunica media compared to $ApoE^{-/-}$ mice. Cyst $C^{-/-}ApoE^{-/-}$ aortae also showed thinning of the tunica media and increased content of intimal SMC. Cyst $C^{-/-}ApoE^{-/-}$ mice also showed dilated thoracic (2.1±0.2 vs. 1.7±0.2 mm, $p<0.002$, n=10/group) and abdominal aortae compared with control $ApoE^{-/-}$ mice, although intimal area, macrophage accumulation, and lipid core size did not differ between these mice. These findings furnish new evidence supporting the importance of cysteine protease/protease inhibitor imbalance in the regulation of arterial integrity and remodeling during atherogenesis and aneurysm formation.

EPIGENETIC, POST-TRANSLATIONAL MECHANISMS IN THE REMODELING OF VASCULAR EXTRACELLULAR MATRIX AND PLAQUE STABILITY.

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Atherosclerotic plaque formation is the result of modulation of smooth muscle cell (SMC) phenotype followed by remodeling of vascular matrix (ECM). Several of the ECM macromolecules are oversynthesized, among them collagen, followed by the selective degradation by uncontrolled excess protease activity from SMC-s and mononuclear cells. Besides the genetic factors involved essentially in lipoprotein metabolism, post-genetic (epi-genetic post-translational) mechanisms were shown to play important roles. These mechanisms are of several order , one of them is the uncovering of matricryptic sites with the proteolytic production of harmful peptides [1]. This is the case with fibronectin (FN) which accumulates in plaque area. FN degradation products were shown to potentiate phenotypic cell transformation, some peptides are strongly pro-inflammatory, inducing cytokines release (IL-1) and up-regulate MMP-type protease expression. Other mechanisms are based on the age-dependent uncoupling of the elastin-laminin receptor (ELR) accompanied by increase of free radical and protease production as a result of overstimulation of ELR by circulating elastin peptides [2]. We could show recently that hyperglycemia plays an important role in the age-dependent uncoupling of ELR. High glucose concentration as in diabetes inhibits ELR-induced Ca^{++}-influx in endothelial cells as shown by patch-clamp experiments and leads to the over production of the cytotoxic peroxynitrite anion. Age-dependent uncoupling of the ELR was shown also to lead to increase of cholesterol production [3], efficiently inhibited in cells of young donors by nanomolar conc. of elastin peptides. ELR in young endothelial cells is coupled to NOS, this coupling is lost in old cells where no more inhibition of cholesterol synthesis is seen by elastin peptides. Such peptides induce vasodilation in precontracted aorta rings by NO production, this effect being lost with aging[4]. Another mechanism based on glycosamnoglycan-CD44 receptor interaction concerns the strong activation of elastase-type endopeptidase (MMP-2 and –9) release in presence of the locally overproduced GAG-s as hyalutonan [5]. –The above-described mechanisms are susceptible to contribute to plaque destabilization and rupture. They clearly show the importance of post-transcriptional mechanisms such as uncontrolled proteolytic activity, receptor uncoupling accompanied by increased free radical and protease production and also hyperglycemia in plaque remodeling, stability and rupture.

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CYCLIC AMP-MEDIATED INHIBITORY REGULATION OF RAC ACTIVATION AND CELL MIGRATION BY THE EP2 SUBTYPE OF PROSTAGLANDIN E2 RECEPTOR IN VASCULAR SMOOTH MUSCLE

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Vascular smooth muscle cells express the EP2 subtype of prostaglandin E2 (PGE2) receptors, which mediates smooth muscle relaxation through cyclic AMP. We found that stimulation of EP2 also induces inhibition of chemotaxis of vascular smooth muscle cells toward platelet-derived growth factor (PDGF). Concomitantly, PGE2 inhibited PDGF-induced activation of the Rho-family small GTPase Rac, which serves as a molecular switch to regulate cell motility. We further investigated molecular mechanisms underlying the negative regulation of Rac and cell migration, using CHO cells that overexpress EP2. In CHO cells, Insulin like growth factor-I (IGF-I) directed chemotaxis, which was dependent on Rac. PGE2 dose-dependently inhibited IGF-I-induced chemotaxis and Rac activation in EP2-overexpressing cells, but not vector-control cells. The inhibitory effects of PGE2 were potentiated by isobutylmethylxanthine, and was mimicked by forskolin and dibutyryl cyclic AMP. In addition, forskolin and dibutyryl cyclic AMP inhibited IGF-I-induced membrane ruffling, which is a typical actin-cytoskeletal change mediated by Rac. The inhibitory effects of PGE2 on chemotaxis and Rac were reversed by the specific cyclic AMP-dependent protein kinase A inhibitor Rp-cAMPS. Either PGE2 or forskolin did not inhibit membrane ruffling induced by the constitutively active Rac mutant V12Rac. PGE2 did not inhibit Rac activation or membrane ruffling induced by the constitutively active Tiam1, a guanine nucleotide exchange factor for Rac, making unlikely the possibility that cyclic AMP stimulates a GTPase-activating protein for Rac. In addition, PGE2 did not inhibit IGF-I-induced tyrosine phosphorylation of IGF-I receptors or Insulin receptor substrate-1, or phosphatidylinositol 3 kinase activation. These results indicate that EP2 mediates Rac inhibition through cyclic AMP and protein kinase A, thereby inhibiting membrane ruffling and chemotaxis. The site of action of the cyclic AMP pathway is suggested to involve inhibition of a guanine nucleotide exchange factor for Rac.

APOPTOSIS INDUCED BY 7-KETOCHOLESTEROL ACCELERATED IN SMOOTH MUSCLE CELLS OBTAINED FROM OLETF RATS

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We reported that 7-ketocholesterol, one of oxysterols accumulated in atherosclerotic lesion, induced apoptosis of human smooth muscle cells[1,2]. This finding might indicate that 7-ketocholesterol make atherosclerotic plaque unstable. Diabetes mellitus is very important risk factor for myocardial infarction, by accelerating atherosclerosis of coronary. But, the mechanism of plaque rupture is not fully understood. We hypothesized that SMC exposed under diabetic condition for a long time might increase sensitivity to 7-ketocholesterol. To clarify the effect of 7-ketocholesterol on apoptosis of SMC exposed under diabetic condition for a long time, we investigated the difference of sensitivity to 7-ketocholesterol between SMC obtained from the Otsuka Long-Evans Tokushima fatty rat (OLETF), which is a model of type 2 diabetes associated with characteristics of mild obesity and late spontaneous onset of hyperglycemia, and Long-Evans Tokushima Otsuka rat (LETO) which is used as age-matched control for OLETF rat. The outgrowth number and the proliferative potential were higher in SMC from OLETF than LETO. When 7-ketocholesterol was added to SMC, the amount of fragmented DNA significantly increased in SMC from OLETF compared to LETO. From theses findings, we hypothesized that SMC recognized high proliferative potential might be more sensitive to 7-ketocholesterol. Then, to clarify the effect of cell cycle on apoptosis induced by 7-ketocholesterol, in SMC incubated without fetal bovine serum or with PDGF-BB, the amount of fragmented DNA induced by 7-ketocholeaterol were studied. Under incubation without fetal bovine serum, the amount of fragmented DNA induced by 7-ketocholesterol significantly decreased. By the addition of PDGF-BB, the amount of fragmented DNA induced by 7-ketocholesterol significantly increased. These results suggested that apoptosis of SMC induced by 7-ketocholesterol could be accelerated when SMC showed high proliferative potential.

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DIFFERENT EXPRESSION OF APOLIPOPROTEIN (a) ISOFORMS IN SERUM AND IN CAROTID PLAQUES

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The accumulation of lipoprotein(a) [Lp(a)] has been demonstrated in arterial walls of human coronary and cerebral vessels; cardio and/or cerebrovascular risk are associated with high Lp(a) levels and low molecular weight apolipoprotein(a) [apo(a)] isoforms. The aim of this study was to evaluate the expression of apo(a) isoforms present in the atherosclerotic plaques of patients who underwent carotid endarterectomy for severe stenosis. **Material and Methods**: 112 patients, 77 males and 35 females were subjected to investigation. A control group, 37 males and 17 females was selected on the basis of total absence of carotid stenosis or other vascular diseases. Patients and controls were over 60 years old. In all subjects, lipids, apoprotein parameters (A-I, A-II, B Lp(a)) and fibrinogen were determined. Apo(a) phenotyping was performed both in serum and in carotid plaque extracts by agarose gel electrophoresis and Western blotting procedure. Apo(a) size was calculated utilizing a commercial standard as reference for the number of Kringle IV repeats ; the relative front and proportion of apo(a) isoforms were determined by densitometric scanning. Patients and controls were analyzed utilizing a KIV repeats cut-off of 22 for the principal isoform (i.e. the most expressed in serum). **Results**: the groups of patients with KIV 22 as the principal isoform in serum had significantly higher Lp(a) concentration both in serum and in plaque extracts compared to groups with KIV>22 (males: 42,2 vs 18,8 mg/dl, $p<0,001$ in serum and 239 vs 144 μg/g, $p<0,05$ in plaques; females: 49,6 vs 24,1 mg/dl, $p<0,01$ in serum and 212 vs 115 μg/g, $p<0,01$ in plaques). In double band phenotype serum the larger apo(a) isoform representing the principal band was 86,5% in males and 76,5% in females, whereas in the carotid plaques it was observed to be only 17,3% and 28,6% respectively. A significantly higher expression of low molecular weight (L) respect to high molecular weight (H) apo(a) isoforms was observed in plaque compared to serum as evidenced by their L/H ratios in both sexes. In all groups of patients the mean number of KIV repeats of principal isoform in plaque was always significantly lower than in serum. **Conclusions**: In carotid plaques the lowest molecular weight apo(a) isoform is always more expressed than the highest one independently of any possible combination of apo(a) isoforms and/or Lp(a) level in serum.

NATURAL HISTORY OF ATHEROSCLEROSIS AND THE TISSUE DIAGNOSIS OF SUSCEPTIBILITY TO FIBROUS PLAQUES

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Fibrous plaques (FP) represent advanced-stage lesions of atherosclerosis (AS) and are the immediate precursors of vulnerable plaques. The various types of FP have been previously described; however, their quantitative extent has only been partially described. In this report, we characterize the extent of FP on the intimal surface of three arteries: the carotid, coronary, and aorta from populations with high and low risks of CHD stratified according to the presence or absence of CHD and associated diseases. We analyzed the distributions of published values for percent intimal surface extent of FP (raised lesions) in carotids of 566 men, ages 22 to 69, necropsied in 1960-1965, and evaluated for FPs' percent extent by standard protocols (1). The carotid FP lesions originated, rated at 0% extent, about age 28-29 in the high risk men, and about age 34-38 in the low risk men. All subject groups showed continuously graded increases of extent from FP origins, with a sharply greater increase in each group's upper quintile compared to the lower quintiles. The FP maximum intimal surface extent of 80% occurred in the highest risk group, thus defining the carotid's *in vivo* maximum capability for the extent of surface involvement. Individuals with near maximum extents of FP occurred at ages 40 to 69 in all risk groups studied, indicating that highly susceptible individuals occurred in all groups independent of age and their group's risk rank. The FP in coronary arteries (N=497) and aortas (N=82) had similar general characteristics. We introduce normalized scores for susceptibility to FP that encompass the full dynamic range of carotid FP's natural development in the pre-statin era. Artery tissues form the foundations for a lesion-based index of true susceptibility to AS FP, and for the *in vivo* direct measurement of an individual's true susceptibility to FP using clinical imaging techniques.

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INHIBITION OF RAT SMOOTH MUSCLE CELL PROLIFERATION BY NEW POTENTIAL TOPOISOMERASES INHIBITORS

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Accumulation of smooth muscle cells (SMC) of the arterial wall in response to local injury is an important aetiologic factor of vascular proliferative disorders such as atherosclerosis and restenosis after angioplasty. Thus a rational approach to reduce the smooth muscle in the vessel wall is to target intracellular mechanisms regulating cell proliferation active during the progression stage of lesion development. DNA topoisomerase I and II constitute a class of nuclear proteins involved in DNA replication that catalyze the passage of individual DNA strands or double helices through one another. This reaction is known to be required for S phase transition of the cell cycle during cellular duplication, and the topoisomerase I inhibitor topotecan has been previously shown to effectively inhibits SMC proliferation and migration. On the basis of this premises the aim of this study was to develop new pharmacological inhibitors of topoisomerase II and study their antiproliferative effect on rat SMC. 18 different compounds, either chemically synthesized or extracted, were tested. 8 compounds show a significant antiproliferative activity. In particular 3 of them (UR1-12, UR5-03 and UR5-08) were able to interfere with SMC proliferation with IC_{50}-values of 5.98, 4.41, and 1.61 μM, respectively. Cell cycle analysis show that incubation with UR1-12, UR5-03 and UR5-08 leads to the accumulation of cells at G2/M phase of the cell cycle in a concentration-dependent manner. Future studies will be performed to directly investigate the inhibitory activity of the tested compounds on topoisomerase II activity in vitro, and their action on SMC proliferation after perivascular manipulation of rabbit carotid artery.

SUBSTRATE SECRETED BY ADIPOCYTES PROMOTE THE FORMATION OF CAPILLARY-LIKE STRUCTURE IN CULTURED VASCULAR ENDOTHERIAL CELLS

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Adipose tissue is thought to play a role of angiogenesis, but the mechanisms are not fully understood. Furthermore, adipose tissue is considered to secrete multiple cytokines and growth factors. We hypothesized that some of the adipocyte-derived cytokines were considered to play an important role of angiogenesis. In this study, the formation of capillary-like structure of cultured human umbilical venous endotherial cells (HUVECs) was investigated using the supernatant of the mature 3T3-L1 preadipocyte. HUVECs were embedded in type I collagen gel. The lengths of capillary-like tubes of the HUVECs were quantified by Scion Image software using the ratio to control. The supernatant of the mature 3T3-L1 preadipocyte promoted the formation of neovascular (903±102%). The result suggested that the substrate secreted by adipocytes could promote the angiogenesis. Next, to clarify the important angiogenetic factor secreted from 3T3-L1 preadipocytes, RNA interference of 3T3-L1 preadipocytes was performed by 100nM small interfering RNAs (siRNAs). In addition of the supernatant of 3T3-L1 preadipocytes incubated with the siRNAs, the lengths of neovascular of the HUVECs in type I collagen gel were quantified. The siRNA for murine vascular endotherial growth factor (VEGF) and hepatocyte growth factor (HGF), which were considered to promote the angiogenesis strongly, was used for RNA interference. Addition of siRNA for VEGF resulted no significant changes them. Addition of siRNA for HGF suppressed the formation of neovascular promoted by the supernatant of 3T3-L1 preadipocyte (376±56%). Our results indicate that the supernatant of adipocyte could promote the formation of capillary-like structure and HGF might be an important factor for angiogenesis as the adipocyte-derived cytokines.

ROLE OF ERYTHROCYTE AGING IN THE CORONARY ATHEROMA PROGRESSION

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In the recent years it has been reported that free oxygen radicals play an important role in the pathogenesis of several degenerative diseases, including cardiovascular disease. The aim of this study, was to evaluate *ex vivo* if the erythrocyte aging and degeneration processes, e.g. microvesiculation were related to coronary atheroma progression. To test this hypothesis, erythrocytes and plasma from 13 patients with coronary lesions (CL) were studied. Moreover, 10 age-matched healthy donors and 11 patients with Chronic Obstructive Pulmonary Disease (COPD) were used as controls. In particular, the following parameters were considered: i) expression of surface molecules associated with the clearance of senescent red blood cells (RBC), e.g. glycophorin A (GA) and phosphatidylserine (PS); ii) expression of band 3 (anion channel molecule involved in RBC homeostasis maintenance); iii) intracellular redox balance; and iv) plasma concentration of proinflammatory cytokines and Annexin V. Preliminary results indicated that in patients with CL a high percentage of altered erythrocytes was observed and that these alterations were accompanied by: a) oxidative imbalance; b) rearrangement of band 3 expression; and c) a significant decreased expression of GA. No significant changes were observed in RBC PS expression. Importantly, increased levels of proinflammatory cytokines and Annexin V were detected in plasma from CL patients. In conclusion, considering that the erythrocyte aging involves loss of GA, cholesterol and cytoskeleton elements (e.g. spectrin) by a "blistering phenomenon" and that trace of GA, iron and cholesterol were found in coronary lesions (Frank D et al., 2003), we hypothesized that the aging of RBC can contribute to CL progression.

THE CELLULAR MECHANISMS INVOLVED IN THE VASODILATOR EFFECT OF NEBIVOLOL ON THE RENAL ARTERY

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Nebivolol is known as a highly selective β_1 adrenergic receptor antagonist. Based on the reported vasodilator effect of nebivolol we examined the cellular mechanisms by which the drug induces renal artery vasodilation, an issue of potential relevance for high blood pressure condition. To this purpose myograph and patch-clamp techniques were used. Small mouse renal arteries were placed in the myograph chamber and after establishing the optimal concentration for the vasodilator effect of nebivolol (50 μM) the arteries were further investigated to assess a potential contribution of nitric oxide (NO) and of Ca^{2+} ions to the nebivolol induced effect, by exposing the arteries to the specific inhibitors such as N^G-nitro-L-arginine methylester (L-NAME, 100 μM), EGTA (4 μM) and thapsigargin (1μM). The expression of NO synthase was evaluated by the Western-blot technique. By both myograph and patch-clamp techniques applied on intact renal artery we investigated the role of β_2- adrenergic receptors, of myoendothelial junctions and of Ca^{2+} activated K^+ channels in the vasodilatory effects of nebivolol, using 100 μM butoxamine, 40 μM 18 beta-glycyrrhetinic acid, 1 mM tetraethylammonium, and 100 nM iberiotoxin, respectively. The results showed that the cellular mechanisms of the vasodilator effect of nebivolol on the renal artery entails (i) activation of the endothelial β_2 adrenergic receptors, (ii) participation of $[Ca^{2+}]_i$, (iii) increase in NO and eNOS, and (iv) activation of Ca^{2+} activated K^+ channels. The cellular mechanisms underlying vasodilator effect of nebivolol on the artery explain the favorable effect of this drug in hypertension.

HYPERTENSIVE PATIENTS WITH CAROTID ARTERY PLAQUE EXHIBIT INCREASED PLATELET AGGREGABILITY

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It is still unclear whether platelet aggregability is related to early stage arteriosclerosis. We used a novel platelet counting system by a laser light scattering method to evaluate the relationship between platelet aggregability and carotid artery arteriosclerosis in 112 outpatients with primary hypertension. All subjects underwent common carotid artery ultrasonography, and determination of plaque. Patients with ischemic heart disease, stroke, or who had more than 20% carotid artery stenosis were excluded. In a platelet spontaneous aggregation, more than twice as many platelet small aggregates formed in specimens from the patients with carotid artery plaque (n=49) have shown as in the specimens from the patients without plaque (n=63) ($p<0.01$). More large aggregates were induced by 2μM of ADP and more small aggregates were induced by 0.5μM of ADP or 0.3μM of epinephrine in the specimens from patients with carotid artery plaque than in the specimens from patients without plaque ($p<0.01$). There were no significant differences between the patients with and without carotid artery plaque in sex, age, blood pressure, body mass index, plasma concentration of glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol, lipoprotein(a), high-sensitivity C reactive protein, or fibrinogen. These results showed that hypertensive patients with carotid artery plaque have increased platelet aggregability. A prospective study was recommended to clarify whether the increased platelet aggregability promotes the progression of arteriosclerosis.

PHOSPHOLIPIDS ARE INVOLVED IN PLANT LECTIN INDUCED PLATELET AGGREGATION

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Our previous studies have shown that the phytohemagglutinin (PHA) from the ***Phaseolus Vulgaris*** is capable to induce in vitro platelet aggregation. The PHA binds specific monocacharides: D-Galactose, D-manose, N-acetil-D-galactosamine on the cell surface and is involved in signal transmission.
The aim of this investigation was to determine platelet membrane phospholipid composition and PHA ability to nonspecifically bind membrane phospholipids.
Platelet membrane phospholipid composition: lysophosphatidylcholin (LPC), phosphatidylserine (PhS), sphingomieline (SpM), phosphatidylcholine (PhCh) phosphatidyletanolamine (PEA) and phosphatide acid (PA)) were identified by chromatograpy analysis. Blood samples were obtained from 12 healthy volunteers and 15 patients with hypertension (I-II stage, WHO classification, aged 25-58). The effect of PHA on platelet shape was viewed under a phase - contrast microscope.
The investigation revealed that membrane phospholipid composition in hypertension patients platelets was significantly different from those of healthy subjects ($P<0,001$). The PHA was capable to nonspecifically bind some phospholipids: PhS, PhCh and SpM. In the presence of PHA the changes of platelet shape from a smooth disc to an irregular form with multiple finger like projection. Plasma membranes had asymmetric distribution of phospholipids that was lost during cell activation. We suggest that phospholipids are involved in cell activation and signal-transmission. The ability of PHA to bind monosacharides/phospholipids on platelet surface induces the loss of phospholipids asymmetry and may serve as a signal for cell-cell recognition and promotes aggregation.

Lpa, FACTOR VII AND TRIGLYCERIDE IN MI PATIENT AND THEIR RELATION TO RESPONSE TO STREPTOKINASE

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Background: Various studies indicate that elevated level of lipoprotein (a), triglyceride (TG) and F-VII are not only the risk factor of Myocardial Infarction (MI) but also are effective in response to treatment, one of the treatment ways of MI is the use of Thrombolytic drugs such as streptokinase (SK). There are different cross reaction factors that decrease the responsiveness to Thrombolytic therapy. Streptokinase antibody and tissue-plasminogen Activator inhibitor (PAI) are some these factors. Lp(a) is considered as a cross reaction factor because of its structural similarity to plasminogen, so it reduces the rate of response and the necrotic potency. Because of high prevalence of non-responsive to streptokinase in our population, it is very important to find factors cause it. In this study the serum level of Lp(a), F-VII and triglyceride in patients with MI who receive SK and its relation with positive response to treatment have been assessed.
Methods: This cross-sectional study was done on 30 patients referred to Emergency Department that their clinical signs and ECG showed MI. Blood sampling and ECG were done for these patients, then 1.5 and 3 hour after getting SK, ECG was repeated and compared with the first one. The serum level of Lp(a), TG and F-VII of patients with positive response to SK and those with negative response to SK was measured. The results were statistically assessed by T-Student, Man-Whitney testes and by correlation coefficient.
Result: There wasn't any significant difference due to three factors in the group with positive response to SK and the group with negative response to SK. The mean Lp(a) in group with negative response to SK was less than the other group. The correlation of response to SK due to TG and F-VII was less than Lp(a).
Conclusion: The elevated level of Lp(a), TG and F-VII is related with the occurrence of MI. The more higher the serum level of Lp(a), the positive response to SK is reduced, because of interaction effect of Lp(a). Comparing the serum level of F-VII and TG with Lp(a) showed that measurement of LP(a) is more important regarding positive response to treatment. Comparing the mean Lp(a) in the community and in patients with MI showed that in patients with MI, Lp(a) level is more higher than normal level. So more attention should be paid to it as a risk factor.

D-DIMERS, FIBRINOGEN AND SERUM LIPIDS IN HYPERTENSIVE PATIENTS.

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Serum lipids have a crucial role in the atherosclerotic plaque formation; D- dimers and fibrinogen are main components of the thrombosis mechanism. These parameters are compared in hypertensive patients and normotensive persons.
73 hypertensive patients (blood pressure >140/90mmHg), were studied (31 men, 42 women) and were compared with 35 normotensive persons (blood pressure ≤ 130/80mmHg). Mean age of all participants was 65±13 years. The following parameters were measured: blood pressure, D-dimers, fibrinogen, serum cholesterol, serum triglycerides. The measuring techniques for D-dimers and fibrinogen were Turbiquant ant Multifibren-n respectively and phasmatometric for the serum lipids.
The results for hypertensive patients and for normotensive persons were respectively: D-dimers = 444±271 – 246±100 μg/L, fibrinogen = 3.87±1.11 – 3.40±0.61 gr/L, serum cholesterol = 217.6±53.2 – 220.1±50.3 mg/dL, serum triglycerides = 127.8±63 – 143.7±65 mg/dL.
From the above measurement is concluded that the elevation of the arterial pressure is followed by a parallel increase of D-dimers ($t=3.59$ with 106d.f, $p< 0.005$) , fibrinogen is increased in hypertensive patients ($t= 2.83$ with 106df, $p< 0.005$). The lipid profile shows no statistically significant difference between the two groups.

RBL-2H3 /NFAT-RE LUCIFERASE CELL LINE: A NEW WAY TO STUDY GPVI RECEPTOR ACTIVATION

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The response of platelets to vessel wall injury is a primary event in arterial thrombosis.
Platelets respond to vessel wall injury via activation of the surface receptor Glycoprotein VI (GPVI), which accelerates the thrombotic response through release of granule contents and activation of platelet integrins.
GPVI, which represents a relevant antithrombotic pharmacological target, is a platelet membrane protein constitutively associated with the Fc Receptor γ chain (FcR γ). Upon activation by Collagen, the specific ligand, GPVI induces a kinase cascade, resulting in the stimulation of PLC-γ 2 which leads to cytosolic Ca^{++} increase.
Zheng et al. (.J.Biol.Chem 2001; 276: 12999-06) demonstrated that the heterologous expression of GPVI in RBL-2H3 cells (rat basophilic leukaemia cell line), which endogenously express the FcR γ chain, is able to confer both signalling and adhesive responses to the high affinity GPVI ligand: convulxin (CVX), a snake venom protein, initially used to biochemically purify the receptor.
Aim of this study was the configuration of a cell based assay suitable for the identification of specific GPVI antagonists. RBL-2H3 were permanently transfected with an expression vector for human GPVI and with a reporter vector expressing Luciferase under the control of NFAT responsive elements. The NFAT transcription factors (Nuclear Factor Activated T cells), which play an essential role in immune response gene expression, are in fact activated by stimuli which increase free cytoplasmic Ca^{++}.
A four hour incubation of GPVI-NFAT-RE Luciferase–RBL2H3 in the presence of CVX results in a very strong (up to 100 fold) Luciferase stimulation.
This cell based system represents a reliable, highly sensitive and very fast method for the identification of GPVI antagonists.

EXCHANGE OF TRANS UNSATURATED FATTY ACIDS BETWEEN ADIPOSE TISSUE AND ATHEROSCLEROTIC PLAQUE STUDIED WITH ARTIFICIAL NEURAL NETWORKS

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The aim of this work was to search for correlations between the content of CLA in the atherosclerotic plaque (AP), adipose tissue (AT), and plas-ma and to determine whether CLA modify the phagocytotic activity of human peripheral blood monocytes/macrophages. Neural networks (NN) were applied to analyze the dynamics of exchange of fatty acids (FA). Advanced APs and small samples of abdominal subcutaneous AT were obtained. Total lipids were extracted using chloroform/methanol conta-ining 0.01% butylated hydroxytoluene as antioxidant and were saponi-fied with 2% KOH. FAs were methylated with boron trifluoride-metha-nol, extracted using hexane, and separated in a gas chromatograph (Per-kin-Elmer 8500) equipped with a flame ionization detector, split-splitless injector, and 105 mcapillary column with RTX 2330 (Resteck Co.). FA methyl esters were computer-identified after a temperature-programmed run against retention times of standards and an electronic integrator was used to measure peak areas. Comparisons were done with the Statistica STNN software package. In the present work, NN were created with the Intelligent Problem Solver. Quantification of monocyte phagocytotic ac-tivity was done with Phagotest (Orpegen Pharma). AP content of CLA isomer appears to depend on AT and blood levels. Accumulation of trans10, cis12 CLA in the AP was accompanied by falling levels in AT. The concentration of this CLA in plasma seemed not to affect this process. Conversely, higher content of cis9, trans11 CLA in AT was associated with accumulation in the AP. Plasma concentrations of this isomer were the prime determinant of AP levels. Both CLA studied by us modified phagocytotic activity of human blood monocytes. Using artifi-cial NN, we were able to confirm an intense exchange of two CLA iso-mers between AT, AP and plasma. Uptake of CLA by the AP appears to depend on its content in AT and concentration in plasma. Changes in the phagocytotic activity of monocytes observed by us could be important for the accumulation of lipids in macrophages of the AP.

INSULIN AND PPARγ STIMULATION OPPOSE POTENTIALLY ATHEROGENIC FATTY ACID-INDUCED ALTERATIONS IN GENE EXPRESSION AND STRUCTURE OF HUMAN ARTERIAL SMOOTH MUSCLE CELL PROTEOGLYCANS

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In insulin resistance and type 2 diabetes arterial cells are exposed to increased plasma non-esterified fatty acids (NEFA). We found that high concentrations (800 μmol/l) of albumin-bound linoleate (LO) upregulate the core protein mRNAs of the major extracellular matrix proteoglycans (PGs) secreted by human arterial smooth muscle cells (hASMC) in culture (versican>biglycan>perlecan).Versican, a PG that can carry up to twelve chondroitin sulfate (CS) chains and the most abundant PG of those secreted by hASMC in culture, can undergo differential splicing. Three variants have been described differing in length and number of CS chains attached. Here we also examined the mRNA for the splice isoforms under the culture conditions. To abrogate the LO-dependent changes on mRNA insulin levels above 1 nmol/l were required or the use of an insulin sensitizer as the PPARγ agonist rosiglitazone (10μmol/l). In addition, high LO increased the mRNA levels for CS-synthase and CS-6 and -4 sulfotransferases, enzymes required for CS- glycosaminoglycan (GAG) synthesis. These changes were accompanied by an increase of the assembled CS-rich PGs secreted into the media. Smooth muscle cell matrix PGs containing CS chains appear to contribute to the retention of apoB-lipoproteins in the arterial intima, a key step in atherogenesis. We found that GAGs secreted by LO-treated cells bound human LDL more efficiently than GAGs from control cells, apparently caused by higher sulfation of the GAGs. Insulin and rosiglitazone inhibited the LO-induced increase in LDL affinity. If present *in vivo* the effects of high NEFA on smooth muscle cells could predispose to atherogenesis, especially when the physiological actions of insulin are compromised.

REGULATION OF GLUCONEOGENESIS BY HUMAN ADIPONECTIN IN HEPATOMA CELLS

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Adiponectin plays important roles in regulating insulin sensitivity and atherogenesis. Adiponectin levels are inversely correlated with the degree of obesity and insulin resistance in human. Murine adiponectin has been shown to suppress glucose production and enhance insulin action in rodents. Human adiponectin protein has 83% similarity to murine adiponectin. The effect of human adiponectin on glucose production in hepatocytes has not yet been reported. In the current studies, we analyzed the effect of recombinant human adiponectin on glucose production in hepatocytes by expressing the protein using adenovirus delivery system. We generated adenovirus encoding human full length or globular domain of the protein. Expression of adiponectin in rat hepatoma H4IIE cells suppressed the expression of the genes encoding glucose-6-phosphate (G6P) and phosphoenolpyruvate carboxykinase (PEPCK), rate limiting enzymes in glucose production pathway. Furthermore, expression of either full length or globular domain of adiponectin results in reduction of glucose production from lactate and pyruvate in H4IIE cells. Purified recombinant human adiponectin also reduces glucose production in H4IIE cells and in cAMP/dexamethasone stimulated rat primary hepatocytes. Human adiponectin appears to function by activating AMP activated protein kinase (AMPK) in hepatocytes.

TRANSGENIC EXPRESSION OF RESISTIN INDUCES IMPAIRED GLUCOSE TOLERANCE AND DECREASED FATTY ACID REESTERIFICATION IN ADIPOCYTES OF THE SHR

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Recently, we derived a novel transgenic strain of spontaneously hypertensive rats (SHR) expressing the mouse resistin (Retn) gene under control of the fat specific aP2 promoter. Expression of the resistin transgene in adipose tissue was associated with increased serum free fatty acids (FFA) and muscle triglycerides, impaired skeletal muscle glucose metabolism, and glucose intolerance. Surprisingly, there were no changes in serum resistin concentrations since transgenic resistin was not secreted from adipocytes. These findings raised the possibility that autocrine effects of transgenic resistin in adipocytes lead to an increased release of FFA from fat. The aim of the current study was to further investigate effects of resistin on fatty acid metabolism. Adrenaline stimulated lipolysis in SHR-Retn transgenic rats versus control SHR rats showed significant increase in FFA secretion (0.73±0.05 vs. 0.57±0.04 mmol/L, $P<0.05$) and higher FFA/glycerol ratio (3.27±0.26 vs. 2.11±0.10, $P=0.0005$). The SHR-Retn rats also exhibited significantly decreased expression of the Pck1 gene that encodes phosphoenolpyruvate carboxykinase (PEPCK), a rate limiting enzyme of glyceroneognesis. The current results suggest that autocrine effects of transgenic resistin induce increased FFA secretion, possibly by suppressing glyceroneogenesis and reesterification of FFA in adipocytes.

IMPAIRED GLUCOSE UPTAKE IN L6 CELLS BY HMG-CO-A-REDUCTASE INHIBITION

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Macrovascular disease is the most common cause of morbidity and mortality in type 2 diabetes. Beyond their effects on circulating lipoproteins, statins have been ascribed potentially beneficial pleiotropic actions such as immune modulation or plaque stabilization. In the West of Scotland Coronary Prevention Study, assignment to pravastatin therapy resulted in a 30% reduction in the incidence of diabetes mellitus (DM). Atorvastatin has been shown to slow the progression atherosclerosis and to reduce vascular events in variety of patient populations including individuals with type 2 DM. We here assessed the hypothesis that atorvastatin might positively affect insulin sensitivity.
L6, skeletal muscle cells were treated for 24 hours with various concentrations of atorvastatin. At 1μM, atorvastatin produced an increase by 42 % in 2-deoxy-D-[1-3H]glucose (2-DG) uptake compared to mock cells. Insulin (100 nM) used as a positive control produced an increased 2-DG uptake by 51%. Co incubation of atorvastatin (1μM) and insulin (100nM) resulted in a slight synergistic effect on 2-DG uptake (61%) compared to mock cells. Mevalonate was used to verify that the stimulatory effect of atorvastatin on 2-DG uptake was mediated by inhibition of HMG CoA reductase. Co incubation of atorvastatin (1μM) and mevalonate (0,5mM) blunted the increment in 2-DG uptake from 42% to 17%.
Our results clearly indicate a beneficial effect of atorvastatin on the glucose metabolism in skeletal muscle cells. This raises the possibility that atorvastatin might positively affect insulin sensitivity and might indeed help to prevent the transition from impaired glucose tolerance to manifest type 2 diabetes.

GLYCATION OF HUMAN AORTIC ELASTIN

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Introduction; Elastic components play a pivotal role in tissues by virtue of their properties. With increasing age elastine become brittle, due to modification by glycation (non-enzymatic glycosylation). This process affects its physical properties and physiological functions and is particularly involved in the cardiovascular complications in diabetes. Non-enzymatic glycation of proteins is a consequence of hyperglycemia in diabetes and correlates with aging. The aim of the study was to investigate age-related changes in the glycation of human aortic elastin in healthy subjects by two approaches:
Methods: (1) assessment by fluorescence method of formed in vivo advanced glycation end products (AGEs) of elastins, purified from human aortas, obtained from different age groups; (2) in vitro glycation of elastins from different age groups and investigation of their capacity to form early (by colorimetric nitroblue tetrazolium method) and AGEs (fluorescence method). Human insoluble elastins were prepared from macro- and microscopic unaltered regions of thoracic aortas, obtained from 68 accident victims, distributed in 15 age-groups, using the method of Starcher and Galione. Soluble -elastins were obtained by the method of Partridge et al.
Results: The direct assessment of Maillard reaction related fluorescence in the age groups showed increase of the fluorescence with age. The 'young' elastin had the highest capacity to form both fructosamine and AGEs under glycation in vitro. The glycation of 'old' elastin did not increase markedly during the incubation. These results are consistent with the interpretation that because of its long biological half-life, elastin is susceptible to the slow process of glycation and the following modifications would contribute to the age-related changes of connective tissue. **Keywords:** Elastin; Non-enzymatic glycation; Fructosamine; Advanced glycation end products; Aging

NORMALIZATION OF TRIGLYCERIDE IMPROVES NEFA LEVELS AND INSULIN RESISTANCE IN OBESE, HYPERINSULINEMIC PATIENTS

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Mobilization of fat is much more insulin sensitive than both hepatic glucose production and peripheral glucose uptake. Non-esterified fatty acid (NEFA) metabolism is strongly dependent on the nutritional state, as both mobilization and reesterification of NEFA in the adipose tissue are exquisitely controlled by the insulin levels. We have used a simple, physiology-based model (1) to analyze the effects of different nutritional states on the interplay between insulin, NEFA and blood triglyceride (TG). The model is able to describe normal NEFA levels and levels that are typical for patients with insulin resistance given the insulin and TG concentrations and the energy expenditure. It also describes effects on NEFA metabolism by variations in plasma insulin and TG after a meal. The results show that the combination of decreased insulin sensitivity and increased TG levels affect the postprandial NEFA and adipose tissue dynamics in an adverse way in obese, hyperinsulinemic patients. However, lowering the TG level can decrease the postprandial NEFA levels and postprandial fat gain in these patients due to decrease in the LPL-mediated spill-over. It is therefore beneficial to normalize the TG levels in obese, hyperinsulinemic patients. The analysis shows that the best tissue to target is the adipose tissue, e.g. both NEFA production and NEFA uptake. In conclusion: The supply and removal of NEFA from adipose tissue depends on the insulin level, the TG level and the insulin sensitivity. Normalization of the TG levels in obese, hyperinsulinemic patients lowers NEFA levels, increases postprandial CHO oxidation, and decreases postprandial adipose tissue fat gain.

PIOGLITAZONE REVERSES TRIGLYCERIDE-RICH LIPOPROTEIN-INDUCED INSULIN RESISTANCE IN L6 SKELETAL MUSCLE CELLS BY A PPAR-γ-DEPENDENT MECHANISM

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Pioglitazone, an insulin-sensitizing drug of the thiazolidinedione class, is used clinically to treat type-2 diabetes. Its action is closely associated with binding to peroxisome proliferator-activated receptor (PPAR)-γ, a specific nuclear receptor. We recently demonstrated that postprandial triglyceride-rich lipoproteins (TGRL) are able to induce insulin resistance in L6 skeletal muscle cells. TGRL reduced insulin-induced glycogen synthesis, glycogen synthase activity, glucose uptake and several crucial insulin signaling steps in a dose- and time-dependent fashion.
The aim of this study was to examine whether pioglitazone is capable of reversing TGRL-induced insulin resistance in L6 skeletal muscle cells and, if so, whether the effect of pioglitazone could be reversed by co-incubation with GW9662, a known PPAR-γ antagonist.
TGRL were isolated by zonal ultracentrifugation and the fraction with $S_f > 300$ was used for the experiments. L6 cells were incubated in the absence or presence of pioglitazone for 24 hours, and TGRL at a triglyceride concentration of 40 mg/dl were added to the incubation media for the last 3 hours. To determine whether the observed effects were PPAR-γ-dependent, cells were incubated with pioglitazone in the absence and presence of the PPAR-γ-antagonist GW9662.
As previously shown, incubation of L6 cells with TGRL reduced insulin-induced glycogen synthesis to 60%. Pretreatment with pioglitazone at 5μM reversed this TGRL-induced impairment of glycogen synthesis almost completely. The ability of pioglitazone to reverse the TGRL-induced effect was eliminated by GW9662 at 10μM. Consistent with the changes in glycogen synthesis, TGRL-induced changes in several insulin signaling steps, i.e. GSK-3- and Akt phosphorylation were also virtually completely reversed by the addition of pioglitazone.
In summary, TGRL-induced changes in glycogen synthesis and insulin signaling could be almost completely reversed by the addition of pioglitazone to the incubation mixture. The effect of pioglitazone was abolished by co-incubation with the selective PPAR-γ antagonist GW9662. We conclude that pioglitazone reverses TGRL-induced insulin resistance in L6 skeletal muscle cells by improving activation of the insulin signaling cascade through a PPAR-γ-dependent mechanism.

THE SOFT-DRINK INDUCED INSULIN RESISTANT RAT AND THE METABOLIC SYNDROME

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Introduction: The metabolic syndrome seen in man is related to lifestyle and genetic disposition. Typical characteristics of the disease complex include: impaired glucose tolerance, insulin resistance, hyperinsulinemia, dyslipidemia, and hypertension. For years, the focus has been on fats when discussing possible dietary components. Recently, however, the interest in carbohydrates - and especially refined sugars - has increased. The aim of this study was to characterize the soft-drink induced insulin resistant rat with respect to the clinical characteristics of the metabolic syndrome. **Materials and Methods:** Twenty twelve-week old male Sprague Dawley rats were stratified into two groups after weight: a 'water' group and a 'soft-drink' group. The rats in the water group had free access to food (Purina 5008) and water, whereas the rats in the soft-drink group in addition to food and water also had free access to sucrose enriched water (37% W/V). Study period: six weeks. Endpoints were: caloric intake, body weight and composition, dyslipidemic status, glucose tolerance, and insulin sensitivity. Total and abdominal fat were determined at baseline and at the end of the study using dual energy x-ray absorptiomentry and computed tomography. Glucose tolerance and insulin sensitivity were evaluated using oral glucose tolerance (OGTT) and insulin tolerance (ip-ITT) tests. **Results:** Compare with water controls sucrose: increased caloric intake, body weight gain, total and abdominal adiposity, induced hyperinsulinemia, dyslipidemia, and insulin resistance. **Conclusion:** The soft-drink induced insulin resistant rat develops several characteristics similar to that of the metabolic syndrome seen in man. In contrast to the clinical situation, however, the hyperinsulinemia and dyslipidemia seen in this model might be a direct results of the increased sucrose intake (not peripheral insulin resistance) and de novo lipogenesis (not lipolysis in adipose tissue).

NORMALWEIGHT BUT METABOLIC OBESE SUBJECTS IN POPULATION OF EASTERN POLAND

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The aim of the study was to compare the group of normalweight subjects with concomitant metabolic disorders typical for metabolic syndrome with the group of normalweight subjects without any metabolic disorders. Material and methods: Two-layer drawn was applied. From the groups of 100,000 subjects aged over 35 in Lublin town and 100,000 in the countryside we drew two groups each consisting of 3000 subjects. 66% of chosen subjects were investigated. We performed physical examination, antropometric measurements (height, weight, waist and hip circumferences, thickness of 4 skinfolds) and laboratory tests (blood lipids, oral glucose tolerance test, serum insulin concentration: fasting and 2 h after glucose load. Body mass index (BMI), waist to hip ratio (WHR), insulin resistance index (HOMA) were calculated. The upper cut-off point of BMI for normalweight subjects was 25 kg/m^2 and WHR for visceral obesity was 0,85 for women and 1,0 in men. Diabetes mellitus (DM), impaired glucose tolerance (IGT) were diagnosed according to WHO criteria (1985). Hypertension was diagnosed according to WHO criteria (1996). Hypertriglyceridaemia was recognised above 1,7 mmol/l, hypo-HDL-cholesterolaemia below 1,0 mmol/l in women and below 0,9 mmol/l in men. Metabolic syndrome (MS) was diagnosed according to WHO criteria (1999). Total fat amount was assessed using the Siri method. Everyday physical activity, smoking habits and familiar aggregation of metabolic disorders were also assessed. The control group (CG) consists of 91 subjects with a comparable female/male ratio, age and BMI. Results: The metabolic syndrome was recognised in 91 (7,1%) normalweight subjects. The mean BMI in group with metabolic syndrome and with control group was 23,3 ± 1,4 and 23,0 ± 1,7 25 kg/m^2, and the mean WHR – 0,92 ± 0,07 and 0,88 ± 0,08 respectively. The total fat amount was assessed at 32,23±7,19% in group with metabolic syndrome and at 27±8,12% in control group. The mean fasting glucose concentration in venous blood was 5,18 ± 1,47 and 5,09 ± 0,57 mmol/l and the mean serum fasting insulin concentration was 6,56 ± 2,73 and 5,38 ± 1,77 μU/ml respectively. The venous blood glucose concentration 2 h after oral load of 75 g glucose was 8,33 ± 1,96 and 5,06 ± 0,98 mmol/l and the mean serum insulin concentration was 51,89 ± 37,27 and 21,8 ± 14,07 μU/ml respectively. We assessed the HOMA index at 1,59±0,73 and 1,21±0,41 respectively. 23% of current smokers, We found 61% of never smokers and 16% of ex-smokers in MS group, and 43%, 42% and 14% in CG respectively. 24% of cases in MS group and 35% of cases in CG were professionally active, and 4,3% of cases in MS group and 13% in CG practised sports systematically. 39% of cases in MS group and 77% in CG group were walking every day for minimum 1 hour.
Conclusions: Total fat amount has a better predictive value than BMI for recognising of metabolic syndrome. It appears that familial aggregation of metabolic disorders and smoking does not have a significant influence on prevalence of insulin resistance in non-obese subjects with metabolic syndrome and the most important factor is low physical activity.

ARE LOW HDL CHOLESTEROL AND ELEVATED TRIGLYCERYDES AN EARLY PREDICTORS OF METABOLIC SYNDROME DEVELOPMENT?

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The metabolic syndrome and type 2 diabetes mellitus are both associated with elevated levels of triglycerides and lowered levels of HDL cholesterol. Our own study performed on representative sample of Eastern Poland population allowed to assess the prevalence of metabolic syndrome diagnosed according to WHO criteria on 29%. The aim of the study was to appreciate the predictive values of atherogenic dyslipidemia in normoglycaemic subjects in the period of 5 years long follow up study. The initial study was performed in 1999-2000 on population aged 35 and over selected by double blind drawning. The five years follow up study started in 2004. In both steps of observation we performed clinical (including 3-times blood pressure measurement) anthropometric (hight, weight, waist and hip circumferences, 4 skinfold thickness) and laboratory (oral glucose tolerance test- OGTT, glycosylated haemoglobin, serum insulin, blood lipids, microalbuminuria) investigations. 69 subjects (29 females and 40 males in mean age 54.7± 10.1) with diabetic dyslipidemia choosen from basal population sample underwent the follow up study. The mean value of body mass index (BMI) was in basal study 27.9±3.9 and in follow up study 27.5±3.8 kg/m^2 (NS), waist to hip ratio (WHR)- 0.96±0.13 vs 0.99±0.05 (NS), total fat content calculated from sum of 4 skinfold thickness by Durning formula- 34.3±7.7 vs 34.9±6.9% (NS) respectively. The mean value of systolic blood pressure was 133.5±21.9 vs 133.8±18.0 mmHg (NS) and diastolic blood pressure was 84.5±9.7 vs 80.5±11.9 mmHg ($p<0.05$). The mean value of total cholesterol was 5.93±1.59 vs 6.43±1.39 mmol/l ($p<0.05$), there was no significant differences between mean LDL cholesterol values but we found significant improvment in mean HDL cholesterol value 0.68±0.22 vs 1.34±0.29 mmol/l ($p<0.01$) and mean tryglicerides value 2.77±1.8 vs 2.2±1.57 mmol/l ($p<0.01$). The diabetes mellitus did not developed in any case, impair glucose tolerance apper durning 5 years follow up in 5.8% studied subjects. According our data low HDL cholesterol and elevated tryglicerides in basal study did not result in accelerated risk of development disturbances in carbohydrate metabolism durning 5 years follow up observation.

HIGH PREVALENCE OF METABOLIC SYNDROME IN CHINESE AMERICAN MEN

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People with the metabolic syndrome are at increased risk for cardiovascular disease (CVD). Screening of metabolic syndrome allows early recognition of individuals at risk for developing CVD. **OBJECTIVE:** To examine the frequency and risk factors of metabolic syndrome among the Chinese Americans living in New York City. **METHODS:** This is a cross-sectional health examination survey. Using a standardized questionnaire, blood pressure (BP), body mass index (BMI), waist to hip ratio (WHR) were recorded; serum total cholesterol (TC), high-density lipoprotein (HDL), fasting triglycerides (FTG), and fasting plasma glucose (FPG) were obtained. The definition of metabolic syndrome encompasses three or more of the following abnormalities: WHR >0.9 in men and >0.85 in women, BMI >23 kg/m^2, BP ≥140/≥ 90 mmHg, FPG ≥110 mg/dL, HDL < 40 mg/dL in men and <50 mg/dL in women, FTG ≥ 150 mg/dL. **RESULTS:** Data were available in 469 Chinese American subjects, 64% females. The prevalence of metabolic syndrome in our sample was 26.48%. Frequency of elevated BP, elevated TG, low HDL, and FPG were 38.81%, 39.66%, 19.83%, and 11.30%, respectively. Overall obesity, measured by BMI, was 64% and centrally obesity, measured by WHR, was 56%. When compared between men and women, the prevalence of metabolic syndrome in men was 40%, more than twice as high as the 19% seen among the women (95% CI, 0.13, 0.30 $p<0.001$); increased central adiposity was more prevalent in men than in women (48% and 19%, $p<0.001$); and FPG was significantly elevated in men than in women (19% and 7%, $p<0.001$). **CONCLUSION:** Multiple metabolic disorders are present in the Chinese Americans. Early detection and treatment of hypertension, dyslipidemia, obesity, and glucose intolerance can prevent the progression of diabetes mellitus and CVD.

METABOLIC SYNDROME IN ELDERLY AGE

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The glucose intolerance prevalence is authentically higher at old age, which specifies that the age is important determinant of such conditions prevalence. About 40 % from total number of patients suffering from diabetes make older people (older than 60 years). It is not diagnosticated promptly at about 10% of elderly people, and 20% of them have disordered glucose tolerance and are subject to the increased risk of cardiovascular and other diseases. It is known, that insulinresistance lies in a basis of diabetes of 2nd type, and metabolic syndrome. The objective of this research was an estimation of tiazolidinedions (pioglitazon) application possibility as a treatment for metabolic syndrome and diabetes of 2nd type for elderly patients suffering from metabolic syndrome. We have carried out research on Actos (pioglitazon) medicine application for 46 elderly patients suffering from metabolic syndrome. The body mass index in examined group of patients was within the limits of 24-32, fluctuations of weight from 67 to 130 kg. During the research we controlled indexes of carbohydrate and lipid metabolism, weight and blood pressure level. The positive influence on carbohydrate metabolism indexes is marked on a background of Actos application in the course of 6 months. The level of glycemia fast significantly decreased from 6,2 to 5,6 mmole/l (i.e. on 9,7 %), glycated hemoglobin from 6,7 to 6,0 % (i.e. on 10,5 % comparing to initial level). The triglycerides level decrease was revealed in blood (on 18,4 %). The general cholesterol level decrease was also statistically significant (on 7,6 %). There was no any essential influence on a blood pressure level during the research. We did not mark a mass increase of on a background of tiazolidinedions application in our researches. Actos use by elderly patients improves indexes of carbohydrate and lipid metabolism that allows wide using of the medicine for treatment of metabolic syndrome and diabetes prophylaxis.

METABOLIC SYNDROM IN YOUNG LEAN PATIENTS WITH ESSENTIAL HYPERTENSION

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Metabolic syndrome (MS) in patients with essential hypertension is frequently associated with overweight and higher age. Therefore the aim of our study was to assess MS in young lean patients with essential hypertension. Subjects: 42 males with non-treated mild hypertension (mean BP 138/71 mmHg), age mean(SE) 22.3(0.6), BMI 22.8(0.3) kg/m^2 was compared to control normotensive (NT) group consisted of 33 healthy males, age 23.6(0.7), BMI 23.1(0.8) kg/m^2 , BP 117/65 (2/2) mm Hg. Methods: Fasting plasma glucose and insulin were used to calculated the index of insulin resistance IR HOMA. Total, HDL and LDL cholesterol and triacylglycerides (TAG) were estimated. Two tests in different days were performed in each subject: oGTT and IVGTT for evaluation of glucose disposal and insulin secretion. Results: Basal and post load plasma glucose concentration did not differ, however basal insulin and C-peptide concentrations were higher in HT than in NT (9.1(0.6) vs. 4.8(0.4), p<0,05, (689(66) vs. 440(32), p<0,01, resp.). Responses of insulin and C-peptide to i.v. glucose load were higher in HT than NT (p<0,001). IR HOMA was higher in HT than in NT group (1.9 (0.2) vs. 1.0 (0.1), p<0.05). There was no significant difference in HDL or LDL cholesterol in HT and NT, however dyslipidemia was observed in 2/3 of HT patients. Three and more risk factors of MS were found in half of HT patients. Conclusions: Young lean hypertensive patients showed normal glucose homeostasis, which is compensated by insulin hypersecretion. They represent another target group in which identification of insulin resistance is important for lifestyle management and for choice of adequate therapy.

A HIGH PROPORTION OF PATIENTS WITH METABOLIC SYNDROME IN FAMILIAL HYPERCHOLESTEROLAEMIA ARE NOT CATEGORISED AS HIGH RISK GROUP

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Background: Both metabolic syndrome (MS) and hypercholesterolaemia (HC) increase the risk of atherosclerosis. Patients with familial hypercholesterolaemia (FH) are at risk of premature coronary artery disease (CAD) but their risk stratification in the presence of MS is unclear. The objective of this study was to examine the risk categorization of patients with primary hypercholesterolaemia with or without MS. ***Materials and Methods:*** A total of 252 subjects, with FH (n=135) and non-familial hypercholesterolaemia [NFH] (n=117) were studied. FH was defined using the Simon-Broome's criteria. MS was defined according to the Adult Treatment Panel III National Cholesterol Education Program (NCEP-ATPIII) and the Asian criteria (AC) which is similar to NCEP ATPIII except for lower waist circumference cut-offs (>80cm in females and >90cm in males). Risk categorization into low, moderate and high risk groups was performed according to NCEP-ATPIII. ***Results:*** The gross prevalence of MS was 24.6% (31.4% in females, 16.5% in males) and 36.5% (43.1% in females and 28.6% in males) according to the NCEP ATPIII and Asian criteria respectively. Among MS by the NCEP ATPIII, 28%, 38% and 34% of the FH, and 49%, 27% and 24% of the NFH were in the high, moderate and low risk categories. Among MS by the AC, 24%, 36% and 40% of the FH, and 44%, 30% and 26% of the NFH were in the high, moderate and low risk categories. Eleven of the 45 (24%) of FH with MS were in the high risk category. Of these, 2/11, 8/11, and 1/11 had diabetes, CAD and 10 year risk of ≥20% respectively. ***Conclusion:*** There is a higher prevalence of MS according to the Asian than NCEP-ATPIII criteria. However, by both MS criteria, a high proportion of FH patients with MS in this population are not categorized as high risk by NCEP-ATPIII guidelines.

THE PREVALENCE OF METABOLIC SYNDROME AMONG NORMALWEIGHT, OVERWEIGHT AND OBESE SUBPOPULATIONS IN EASTERN POLAND.

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The aim of the study was to assess the prevalence of metabolic syndrome in a representative sample of adult population in Lublin Region (Eastern Poland) and the distribution among body weight categories: normal weight (NW), overweight (OW) and obesity (OB). Material and methods: 3782 subjects aged over 35 years were examined. Physical examination, blood pressure measurement, anthropometric measurements (height, weight, waist and hip circumferences, thickness of 4 skinfolds) and laboratory tests (oral glucose tolerance test – OGTT, serum insulin concentration: fasting and 2 h after glucose oral load, blood lipids) were performed. Obesity/overweight, arterial hypertension, diabetes mellitus (DM)/impaired glucose tolerance (IGT) metabolic syndrome (MS) were diagnosed according to WHO criteria. Body mass index (BMI), waist to hip ratio (WHR) and index of insulin resistance HOMA were calculated. Results: we assessed the prevalence of NW, OW and OB at 33,6%, 35,9% and 30,5% respectively. MS was recognized in 759 subjects (20,1% of studied cases): in 7,1% of NW, 18,9% of OW and 35,6% of OB subjects. The mean BMI in NW, OW, and OB groups was 23,1 ± 1,8, 27,7 ± 1,3 and 33,3 ± 2,9 respectively and the mean WHR: 0,92 ± 0,08, 0,96 ± 0,07 and 0,98 ± 0,08 respectively. The total amount of fat in NW group with MS was as high as 31,8 ± 7,7% and was comparable to that assessed in OB group without MS. In OB subjects with MS the mean fat amount we assessed at 36,7 ± 4,5%. The fasting venous blood glucose concentration in NW, OW, OB groups was 5,6 ± 1,5, 6,1 ± 2,1 and 5,0 ± 0,5 mmol/l respectively. The glycaemia 2 h after oral glucose load was 8,3 ± 2,0, 8,9 ± 2,2 and 8,6 ± 2,1 mmol/l respectively. The serum fasting insulin concentration in NW, OW, OB groups was 6,5 ± 2,7, 8,1 ± 6,6 and 10,9 ± 4,9 μU/ml respectively. The mean insulin concentration 2 h after glucose load was 51,9 ± 37,2, 75,5 ± 64,8 and 131,0 ± 111,7 μU/ml respectively. The mean value of HOMA index in NW, OW and OB groups was 1,59 ± 0,73, 2,15 ± 1,11 and 3,22 ± 0,64. We observed different composition of glucose tolerance disorders in MS subjects in three body weight categories: IGT to DM ratio in NW group was 77% vs 23%, in OW group: 60% vs 40% and in OB group 47% vs 53%. prevalence of unknown DM in NW group with MS was 66%, in OW group with MS – 52,4% and in OB group – 33% of cases. Conclusions: We observed the increase of HOMA index due to increase of BMI in subjects with MS. Our results confirm that obesity is suggestive for prediction of diabetes. The cluster of metabolic disorders (hypertriglyceridaemia, hypo-HDL-cholesterolaemia, hypertension) even in normalweight subjects should suggest necessity of search for diabetes.

METABOLIC SYNDROME AS A RISK FACTOR OF CARDIOVASCULAR DISEASES IN A PORTUGUESE SAMPLE POPULATION

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Metabolic syndrome or insulin resistance syndrome is now considered as very important in the pathogenesis of cardiovascular diseases (CVD) and type 2 diabetes. It involves some or all the following disorders/risk factors; hyperinsulinemia, alterations of glucose and lipid metabolisms, abnormalities of coagulation and fibrinolisis, high uric acid blood levels, hypertension and obesity. Though this metabolic syndrome seems to be mainly enhanced by insulin resistance and obesity. As it is known that 15% of Portuguese population have obesity and over 50% excess weight we decided to inquire about this insulin resistance problem among us, and a population sample was considered. It consisted of 118 subjects, both sexes, 49 men and 69 women, 30-69 years old, non diabetic but clinically defined as having cardiovascular problems or any familial risk factor for CVD or type 2 diabetes. In these fasting subjects we quantified glucose, insulin, HDL cholesterol, triglycerides and uric acid using automatic laboratory methods, and we calculated HOMA and BMI. Our results showed in both sexes and through different age groups a great percentage of individuals with values higher than the upper acceptable limit (exception for HDL cholesterol). Based on HOMA (>3) the number of subjects with insulin resistance was high in each age group reaching 40.82% in men and 13.04% in women.
We could even verify that when HOMA or BMI values increase from the first to the fourth quartile the accumulation of the considered risk factors increases as well as the number of those affected.
In conclusion we could prove that HOMA and BMI values seem to enhance the components of metabolic syndrome and consequently the risk of cardiovascular diseases. So the subjects with high values of HOMA and BMI should be considered in cardiovascular disease risk and for appropriate prevention or treatment.

LIPID-MODIFICATION IN PATIENTS WITH METABOLIC SYNDROME AND CORONARY HEART DISEASE

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Introduction. The important role of the metabolic syndrome (MS) has been stated on the way of developing ischemic heart disease (IHD) in recent years. Each component of MS - insulin resistance, hyperinsulinemia, glucose intolerance, hypertension, dyslipidemia, obesity - itself represents risk factors of atherosclerosis. Objectives. The Aim of the present work was management of dislipidemia in patients with metabolic syndrome and coronary heart disease (CHD). Material and Research Design. In the order 481 patients aged 40-60 years with arterial hypertension and complaints of IHD were examined (mean SBP - 155,5 mm/Hg, DBP - 95,4 mm/Hg). After the screening of the lipid profile 370 patients turned out to have dyslipidemia (mean TCHOL - 6,78 mmol/l, HDL - 0,96 mmol/l, LDL - 4,27 mmol/l, Tg - 2,51mmol/l). 234 (63,24%) patients with dyslipidemia manifested normoglicemia (mean pre-prandial GL- 5,11 mmol/l); among of them 40 (17,1%) patients had IHD (examined group without MS). 102 (27,6%) patients with dyslipidemia manifested glucose intolerance (mean pre-prandial - 6,68 mmol/l, postprandial GL - 7,90 mmol/l; MS was evaluated in 92 (90,2%) patients of them (FS insulin >30 mu/ml, mean waist circumference - 98,2cm); 42 (41,2%) patients with MS turned out to have IHD (examined group with MS). Obtained data of investigation were placed in SPSS database. Results. In both study groups to achieve target Lipid levels patients, together with basic therapy, were treated with standard antiatherosclerosis diet and atorvastatine - a lipid lowering drug. In first examine group patients (mean TCHO - 6,25mmol/l, HDL - 0,98mmol/l, LDL - 4,1mmol/l) were treated with atorvastatine at a dose 10-20mg. In II examine group patients (mean TCHO - 7,6 mmol/l, HDL - 0,56mmol/l, LDL - 4,65 mmol/l) were treated with atorvastatine at a dose 20-40 mg. One mouth post treatment initiation in 38 (96,2%) I examine group patients target levels of lipid metabolism were achieved in 2 patients (3,8%) these levels were at an upper border of normal range (TCHO -5,8 mmol/l, HDL - >0,91 mmol/l, LDL - <3 mmol/l). In I examine group patients target levels were achieved in 12 (28,6%) cases no positive changes were observed. Conclusion: In patients with CHD and metabolic syndrome dislipidemia develops more aggressively and needs drug therapy at higher doses.

MEAN BLOOD PRESSURE AND THE RATIO TRIGLYCERIDES/HDL-CHOLESTEROL AS THE VARIABLES THAT CORRELATE MORE STRONGLY WITH THE METABOLIC SYNDROME SCORE.

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Objective: To assess what parameters correlate more strongly with the metabolic syndrome's score.
Patients and methods: Data from 7,258 healthy workers were collected from the annual health revisions. The assessment of the MS was made, based on the modified ATP-III, by the presence of three of the five following components: body mass index (BMI) >28.8kg/m^2 or waist perimeter (WP) > 102 cm in men or >88 cm in women; blood pressure (BP) >130/85 or being under antihypertensive treatment; triglycerides (TG) >150 mg/dl; HDL-cholesterol <40 mg/dl (males) or <50 mg/dl (females); fasting plasma glucose >110 mg/dl or diabetes mellitus (DM) previously diagnosed. The metabolic score was calculated as the total of components that clustered in the same subject. The correlations were evaluated by bivariant correlation and are presented as Pearson's coefficient.
Results: The mean age was 45.4 (8.9) years; 82.4% of them males. The global prevalence of the MS was 10.5% (743 out of 7,258). Mean BP and diastolic BP showed the most strong correlation (r= 0.52; p<0.001) followed by the ratio TG/HDL-c (r=0.48), total TG (r=0.43), fasting glucose (r=0.37), systolic BP (r=0.34), age (r=0.33), al p<0.001. HDL-c was the obtained the only negative correlation (r=-0.25). BMI, LDL-c and pulse pressure showed very weak correlation (r<0.15)
Conclusions: Blood pressure and triglyceridemia play a key role in the clinical characteristics of the MS. The value of HDL-cholesterol is more relevant by the ratio TG/HDL.

GENDER DIFFERENCES IN THE COMPONENTS OF THE METABOLIC SYNDROME AMONG CARDIOLOGICAL OUTPATIENTS.

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Objective: To assess the differences in the components of the metabolic syndrome (MS) among cardiological outpatients.
Patients and methods: Data 1,000 consecutive patients attending to a outpatient universitary cardiological clinic were collected. The assessment of the MS was made, based on by the on the modified ATP-III, by the presence of three of the five following components: body mass index (BMI) >28.8kg/m^2 or waist perimeter (WP) > 102 cm in men or >88 cm in women; blood pressure >130/85 or being under antihypertensive treatment; triglycerides >150 mg/dl; HDL-cholesterol <40 mg/dl (males) or <50 mg/dl (females); fasting plasma glucose >110 mg/dl or diabetes mellitus (DM) previously diagnosed.
Results: Analytic parameters were available in 806 patients (80.6%); 72.4% males; mean age 55.4 (13.2). The global prevalence of the MS was 29.5% (239 out of 806). The prevalence was higher in women than in men (34.5% vs 27.7%; p=0.059). Age, BMI and the other components of the MS were equally prevalent in both sexes, but women had a significantly higher prevalence of increased WP (80% vs 47%), low HDL-c (65% vs 47%) and hypertension (51.7% vs 41.7%). The prevalence of MS increased parallel to age and was higher in elderly women in the four cuartiles of age. Gender differences in the prevalence of MS only reached statistical significance in the group of age that ranged from 60 to 75 year (50% in women and 32.6% in men; p=0.03).The prevalence of MS in hypertensive outpatients was 51.3%, and tended to be higher in women (52.1% vs 49.1%; p=0,2). Patients with DM or impaired fasting glucose (IFG) had the highest prevalence of MS (69%), being much higher in women than in men (89.3% vs 61.1%; p<0.001).
Conclusions: The MS is highly prevalent among cardiological outpatients. Increased waist circumference, low HDL-cholesterol and hypertension are the most prevalent traits, especially in women. Hypertensive or diabetic outpatients have a higher prevalence of MS, and most of diabetic women have MS.

NAFLD, INSULIN RESISTANCE AND INFLAMMATION

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Background. The pathogenesis of NAFLD involves factors such as insulin resistance and inflammation. The genetic aspects of NAFLD are still undefined. However, it is known that PC-1 K121Q polymorphism has a strong association with insulin resistance and IL-6 G174C with proinflammatory phenotype. **Aim.** To evaluate the prevalence of PC-1 K121Q, IL-6 G174C and their possible association with severity of steatosis and extent of liver damage. **Methods** We studied 100 healthy controls and 123 subjects with NAFLD. Diagnosis was based on ultrasonographic "bright liver", absent/low alcohol consumption (<20 g/die in women, <30 g/die in men) and exclusion of other aetiologies of liver diseases; 64 NAFLD subjects underwent liver biopsy. Extent of steatosis, grading and staging were classified according to Brunt and according to Matteoni. Genetic polymorphisms were evaluated by Restriction Fragment Length Polymorphism (RFLP) technique. HOmeostatic Metabolic Assessment-Insulin Resistance (HOMA) and Quantitative Insulin Sensitivity Check Index (QUICKI), were used to evaluate insulin resistance (IR) and sensitivity. **Results** Prevalence of PC-1 K121Q was comparable in controls and NALFD patients: n NAFLD group "grading" was higher in 121Q vs K carriers (grade 2 : 42% vs 17%) even though the difference did not reach statistical significance; furthermore 72% of 121Q variant carriers had a type 3-4 NAFLD compared to 56% in KK. The prevalence of IL-6G174C was higher in the NAFLD group as compared to controls: GG 24%vs 47%, GC 59% vs 45%, CC 17% vs 8%(P=0.002). NAFLD patients with higher "grading" and "staging" on liver biopsy were more frequently C carriers (CC+GC) as compared to G carriers (GG) although the differences did not reach statistical significance [grade 2: 29%vs 9% in (NS)] [Stage 2: 24%vs10% (NS)]. **Conclusions** Polymorphisms PC-1K121Q and IL-6G174C seem to be associated with greater inflammatory activity and more advanced type of NAFLD suggesting that they might be involved in its progression.