DISTINCT EFFECTS OF LXRα and LXRβ ON HDL AND TRIGLYCERIDE METABOLISM IN MICE

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The nuclear receptor LXR regulates multiple genes involved in lipid metabolic pathways such as ABCA1, which catalyzes HDL formation, and SREBP-1c, a master regulator of lipogenic genes. Two isoforms with different tissue distribution exist, LXRα (NR1H3) and LXRβ (NR1H2). Synthetic compounds that activate both LXR subtypes raise HDL and decrease atherosclerosis in mice. Unfortunately, these compounds also cause fatty liver, likely through SREBP-1c-dependent stimulation of hepatic triglyceride synthesis. It is conceivable that dissociation between beneficial and detrimental effects could be obtained by an agonist specific for either of the isoforms but no such compound has been described. Here we show that an LXRα specific agonist (LXRα/β binding: 80 nM/>50 μM) raised liver and serum triglycerides comparably to dual agonists without any additional HDL benefit; similar results were obtained when treating LXRβ-deficient mice with an LXRα/β dual agonist. In contrast, treatment of LXRα-deficient mice with a dual agonist led to significantly increased HDL levels with modest effects on serum and liver triglyceride levels (LXRα- and LXRβ-deficient mice were obtained from Deltagen). These results suggest that most of the lipogenic effects of LXR agonists are mediated through LXRα whereas the HDL-raising effect is mediated both by LXRα and LXRβ. Specific agonists of LXRβ may thus be viable candidates for drug development.

THE CETP-INHIBITOR TORCETRAPIB RAISES HDL AND PREVENTS AORTIC ATHEROSCLEROSIS IN RABBITS.

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Epidemiological data indicate that low levels of HDL-cholesterol are associated with an increased risk of CHD. Individuals deficient in cholesteryl ester transfer protein (CETP) activity have elevated HDL levels, and consistent with that finding, inhibition of CETP in animals and in man leads to increased HDL-C levels. However, elevation of HDL in experimental animals has not always proven anti-atherogenic. To investigate whether the elevation of HDL via CETP inhibition prevents or retards atherosclerosis, we tested torcetrapib, a potent CETP inhibitor, currently in Phase III clinical trials, in a cholesterol-fed rabbit model of atherosclerosis. Torcetrapib was administered via diet admixture (0.15%) to New Zealand white rabbits (n=19-20) fed 0.2% cholesterol, 10% coconut oil for 16 weeks. Animals were bled periodically for the determination of plasma lipoprotein levels and CE transfer activity. As a result of feeding the atherogenic diet, non-HDL-C levels in both control and torcetrapib-treated groups gradually increased over the duration of the study with no difference apparent at study's end (645±105 mg/dl vs. 690±104 mg/dl mean +/- SEM, respectively). In the group of animals fed torcetrapib, CE transfer activity was inhibited by 70-80% at all time points examined, and HDL-C was elevated immediately at week 1 (200±15 mg/dl vs. 57±4 mg/dl in controls) and remained elevated throughout the treatment period (207±32 mg/dl vs. 57±6 mg/dl at 16 wk). Aortic atherosclerosis was assessed from en face preparations of unstained aortic tissue perfusion-fixed with 10% formalin in situ. The percentage of aortic surface covered with lesions was 60% lower in torcetrapib-treated animals (39.8±5.4% vs. 16.4±3.4%, $p<0.001$). Reduction in aortic atherosclerotic area in the torcetrapib-treated group was significantly associated with elevated levels of HDL, but not with non-HDL cholesterol levels. We conclude that the elevation of HDL via CETP inhibition with torcetrapib inhibits atherosclerosis in cholesterol-fed rabbits.

COMPROMISED LCAT FUNCTION LEADS TO INCREASED PROGRESSION OF CAROTID ARTERIAL WALL THICKNESS

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Background. Epidemiological studies have shown that low plasma levels of HDL-c are associated with an increased risk for atherosclerotic vascular disease. It is, however, unclear whether this also holds true for individuals with reduced levels of HDL-c due to mutations in lecithin:cholesterol acyltransferase (LCAT). An unparalleled cohort consisting of 68 carriers of LCAT gene mutations enabled us to address this question. **Methods.** In 9 homozygous carriers of LCAT defects, characterized by complete loss of LCAT function against apoAI-containing proteoliposomes, 59 heterozygous carriers and 74 family controls, we measured lipids, lipoproteins, hsCRP, and mean carotid arterial intima media thickness (IMT). **Results.** Compared to controls, heterozygotes and homozygotes presented with a 40 and 90% decrease in HDL-c respectively ($p<0.001$ for both), as well as a 22% and 337% increase in TG levels ($p<0.001$ for both). Surprisingly, heterozygotes and homozygotes also displayed a 2.4 and 2.1 fold increase in median hsCRP levels compared to controls ($p=0.0001$ and $p=0.631$ respectively). Cross-sectional IMT data plotted against age showed a 0.0030, 0.0053, and 0.0067 mm increase of mean carotid IMT per year in controls, heterozygotes and homozygotes, respectively. Adjusted for age, sex and family, IMT was significantly increased in carriers of LCAT mutations compared to controls ($p=0.0053$). **Discussion.** LCAT gene defects are associated with accelerated arterial wall thickness progression and an increased incidence of vascular events. This suggests that the LCAT protein is actively involved in the protection against atherosclerosis progression and indicates that this key regulator of HDL-c levels might be a promising target for the prevention of vascular disease.

PLEIOTROPIC EFFECTS OF LIPID MODIFYING DRUGS

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A number of observations support the concept that statins exhibit beneficial effects independently of lipid lowering. For example: 1) In clinical trials, statins reduce clinical events faster than other lipid-lowering treatments. 2) In monkeys with dietary hypercholesterolemia and atherosclerosis, statins promote plaque stabilization even when serum cholesterol is "clamped" at a steady level. 3) In humans, statins improve endothelial function even before serum cholesterol lowering can be detected. 4) In clinical trials, anti-inflammatory effects (as measured by C-reactive protein) of statins are largely independent of LDL-cholesterol lowering. 5) Unlike statins, ezetimibe, a cholesterol-lowering agent that acts by preventing cholesterol absorption does not by itself reduce C-reactive protein. 6) In REVERSAL study, progression/regression of coronary atherosclerosis with statin therapy can be attributed to C-reactive protein reduction independently of any lipid reduction. Admittedly, no amount of such circumstantial evidence is convincing enough of a lipid-independent effect until the specific mechanisms can be identified at a molecular level. Recent research has began to elucidate such molecular mechanisms. Cholesterol synthesis leads to LDL production but also fuels isoprenylation that activates the RhoA-GTP signaling molecules and their downstream effector Rho kinase (ROCK). In animals, Rho/ROCK activation results in vascular inflammation, vasoconstriction and atherogenesis, and these processes are inhibited by statins through Rho/ROCK inhibition. The ability of statins to inhibit Rho/ROCK is under investigation in humans.

MODULATION OF CELLULAR CHOLESTEROL EFFLUX BY LIPID AFFECTING DRUGS

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Cellular cholesterol efflux, the first step in reverse cholesterol transport, protects cells from an excessive accumulation of cholesteryl esters or cytotoxic free cholesterol. Three major mechanisms are involved in cellular cholesterol efflux: passive diffusion, the ABCA1 mediated active transport and the SRB1 facilitated diffusion. ABCA1 exerts antiatherosclerotic properties by two main mechanisms: the first is the lipidation of apoA-I in the liver by promoting lipid efflux from hepatocytes, essential for the formation of HDL. The second is the promotion of lipid efflux from macrophages that prevents peripheral tissues from foam cells accumulation. Several compounds have been reported to be able to modulate ABCA1 expression. LXR and RXR agonists and cAMP are very active on this regard. Stimulation of ABCA1 mediated efflux has been reported also for PPAR agonists and Ca antagonists (CA). In Fu5AH rat hepatoma cells, that we demonstrated to express functional ABCA1 upon treatment with LXR/RXR agonists, but not cAMP, we observed that statins induce cholesterol efflux to apoA-I; the effect was reversed by mevalonate or geranyl geraniol. We demonstrated that probucol specifically inhibits cholesterol efflux to apoA-I by impairing the translocation of ABCA1 to plasma membrane. Interestingly, probucol was reported to protect cells from cytotoxic effect induced by free cholesterol. We observed similar results with lipophilic CA. Omega 3 fatty acids have been also reported to inhibit ABCA1 efflux in macrophages. However, in our laboratory we observed that this effect may occurs for most fatty acids and only at very high concentrations. At lower, more physiological concentrations no effect or a stimulatory one could be observed, depending on the fatty acids tested. In conclusion lipid efflux mediated by ABCA1 appears to be modulate by a number of different compounds and may represent a very promising pharmacological target for developing antiatherosclerotic compounds.

DUAL EFFECT OF STATINS ON ABCA1-MEDIATED LIPID EFFLUX FROM MACROPHAGES AND HEPATIC CELLS

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ABCA1 exerts antiatherosclerotic properties by two main mechanisms: the first is the lipidation of apoA-I in the liver, essential for the formation of HDL. The second is the promotion of lipid efflux from macrophages that prevents peripheral tissues from foam cells accumulation. The beneficial effects of statins on the treatment of atherosclerotic cardiovascular disease are in part independent of the cholesterol lowering properties, but the underlying mechanisms are not fully understood. In this work we investigated the ability of pitavastatin to modulate ABCA1-mediated efflux in macrophages and hepatic cells. Pitavastatin (0,1-10μM) dose dependently reduced cholesterol efflux up to 60% from macrophages expressing ABCA1 upon treatment with cAMP, but no effect was detected when the protein was up-regulated by 22OH/cRA. The inhibition in cAMP-pretreated cells was reversed by mevalonate and partially by geranyl geraniol. Importantly, pitavastatin up to 50μM did not affect cholesterol release from cholesterol-loaded macrophages, a cellular model of foam cells. The effect of pitavastatin on hepatic cells was evaluated in Fu5AH rat hepatoma cells, that we demonstrated to express functional ABCA1 upon treatment with 22OH/cRA. In this model the compound induced a 1,6 fold increase in cholesterol efflux to apoA-I; the effect was dose-dependent, reached the maximum at 10μM and was reversed by mevalonate or geranyl geraniol. Similar results were obtained with compactin, suggesting a class-related effect of statins. The present work shows that statins may inhibit ABCA1-mediated efflux in macrophages only when the protein expression is induced by cAMP; the lack of influence in foam cells seems to exclude a potential negative pleiotropic effect. On the contrary, the increase in cholesterol efflux from hepatic cells may help explaining the improvement in HDL plasma profile observed in patients treated with statins.

STATINS INCREASE EXPRESSION OF MACROPHAGE SCAVENGER RECEPTOR CLASS B TYPE I (SR-BI)

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Formation of cholesterol-enriched macrophage/foam cells is an early and critical step in atherosclerotic lesion development. SR-BI, a receptor for HDL, plays an important role in bi-directional cholesterol exchange between cells and HDL particles and in atherosclerotic lesion development. Over-expression of SR-BI reduces atherogenesis, while lack of SR-BI expression accelerates lesion development in pro-atherogenic mice. Statins, inhibitors of HMG-CoA reductase, significantly suppress cholesterol synthesis and reduce the incidence of coronary heart disease. We investigated the effect of pitavastatin (NK-104) on macrophage SR-BI expression. Pitavastatin, and other statins, significantly increased SR-BI mRNA and protein expression in macrophage cell lines. Induction of SR-BI expression by pitavastatin was time- and concentration-dependent and was also observed in mouse peritoneal and human monocyte-derived macrophages. Inhibition of macrophage SR-BI expression by LPS and tumor necrosis factor-α was restored by pitavastatin and inhibitors of NF-kB. Pitavastatin inhibited NF- B DNA binding activity (as determined by EMSA) and inhibition was mediated through regulation of NF-kB p65 and I B-alpha expression. Our data demonstrate a novel effect of statins that could contribute to inhibiting atherosclerotic plaque formation. Supported by the Abercrombie (AMG) and Silberman Foundations (ACN) and a sponsored research agreement with Kowa Ltd. (AMG, DPH).

ROSUVASTATIN REDUCES INFLAMMATION AND ATHEROTHROMBOSIS IN APO E-DEFICIENT MICE

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Statins have been shown to possess several antiatherogenic properties independent of cholesterol lowering in experimental settings. Based on these premises, we investigated the anti-inflammatory and anti-atherothrombotic properties of rosuvastatin in apoE-deficient female mice. Animals were fed a cholesterol-rich (HC) diet containing rosuvastatin (0, 1, 2 or 10 mg/kg per day; n=9 mice per group) for 12 weeks. Treatment with rosuvastatin did not significantly alter either body weight gain or plasma total cholesterol (C) and triglyceride levels compared to control animals. However, rosuvastatin treatment dose-dependently reduced ICAM-1 expression in the aortic valves (V) (up to 40% inhibition, $p<0.01$) and in the proximal segment of the ascending aorta (AA) (up to 50%, $p<0.001$). Similarly, rosuvastatin inhibited VCAM-1 expression in the V (up to 40%, $p<0.01$) and in the AA (up to 35%, $p<0.01$). Moreover, these inhibitory effects of rosuvastatin on the expression of adhesion molecules led to a reduced accumulation of macrophages in the V in a dose-dependent and statistically significant manner (-50%, $p<0.001$). These anti-inflammatory effects were reflected in a dramatic reduction of cholesterol deposition in the entire aorta, both in the free and in the esterified form (free -50%; esterified -70%), with an increase in the ratio between free C/esterified C. Finally, the expression of tissue factor, the most potent prothrombogenic agent, increased after starting the HC diet but was consistently reduced in AA by rosuvastatin treatment (-71%, $p<0.001$) even at the lowest dose. Altogether, the data demonstrate that rosuvastatin has anti-inflammatory and anti-atherothrombotic activities beyond its plasma cholesterol-lowering effect in apoE-deficient mice that could translate in a beneficial effect in atherogenesis.

INCREASED ACTIVATION OF NF-KB AND ERK1/2 AFTER PERMANENT FOCAL ISCHEMIA IS ABOLISHED BY SIMVASTATIN PRE-TREATMENT

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The role of inflammation in ischemic brain damage has been reported in human and in animal models of stroke. The proinflammatory genes are under the control of the transcription factor NF-kB that is also up-regulated in experimental stroke. Clinical and experimental studies showed that statins reduce the incidence of stroke and the size of brain damage suggesting a role of these drugs in neuroprotection. Since statins, besides lipid lowering action, exert also anti-inflammatory activity we sought to investigate the effect of simvastatin treatment on NF-kB regulation and on NF-kB-activating signalling pathways in permanent-MCAO. In order to determine whether simvastatin could interfere with MCAO-induced activation of NF-kB, animals were treated for 3 days with 20 mg/kg of statin before MCAO. Pre-treatment with simvastatin abolished the activation of NK-kB observed in vehicle-treated animals. We have also evaluated the modulation of different signal transduction pathways such as ERK 1/2, SAPK/JNK 46/54 and p38. Under our experimental conditions, the expression of ERK1/2 was enhanced by ischemia and this activation was prevented by prophylactic administration of simvastatin. We conclude that the transcription factor NF-kB and the ERK1/2 pathway are induced by permanent focal ischemia, however, these enhanced activities were abolished by prophylactic simvastatin administration indicating that preventing the induction of cerebral inflammation may lead to neuroprotection.

ROSUVASTATIN INHIBITS POSTPRANDIAL NEUTROPHIL MIGRATION IN PATIENTS WITH PREMATURE CORONARY SCLEROSIS

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In response to chemoattractants neutrophils are recruited, activated and adhere to the endothelium where they are thought to affect endothelial function and the atherosclerotic process via oxidative mechanisms. In healthy subjects, neutrophil count in peripheral blood increases after an oral fat load, reflecting a postprandial pro-inflammatory situation that may be important for the development of atherosclerosis. We studied quantitative and qualitative postprandial neutrophil changes in 20 male premature coronary artery disease (CAD) patients (50±4 years) before and after 4 weeks treatment with rosuvastatin 40 mg/day. Postprandial neutrophil function was investigated *ex vivo* by determining migration of isolated postprandial neutrophils towards interleukin-8, fMLP and C5a as chemoattractants in a fluorescence-based assay. Neutrophils were obtained during standardized oral fat-loading tests (8 hours). Fasting LDL-cholesterol (4.4±1.0mM), triglycerides (2.2±0.9mM) and HDL-cholesterol (0.93±0.23) improved upon treatment (-59%, -31% and +18%, P<0.01 for each). Total plasma triglyceride clearance (TG-AUC) improved by 23% (p<0.005), whereas the incremental TG response (TG-dAUC) was unaffected by rosuvastatin. The maximal neutrophil increase was +14% at T=3h (P<0.01) before and +10% at T=3h (P<0.01) after treatment (ns between tests). Before treatment, neutrophil migration towards all three chemoattractants increased gradually in a time and chemoattractant-dose dependent manner with a 2-fold increased response at T=8h (*P*<0.05 vs T=0h), suggesting postprandial sensitization of neutrophils for these agents. In contrast, migration of postprandial neutrophils was not altered by rosuvastatin treatment compared to fasting neutrophils. The migration towards IL-8 at T=8h before rosuvastatin was related to TG-dAUC (R=0.72, *P*=0.02) and was not related to postprandial oxidative stress generation or the expression of inflammatory markers (CD11b, CD66B, CD18), suggesting a lipid-dependent effect of rosuvastatin on neutrophil migration. In conclusion, in premature CAD patients, rosuvastatin does not affect the postprandial increase in neutrophil counts but inhibits the postprandially increased *ex vivo* migratory capacity of neutrophils. This anti-inflammatory property of rosuvastatin may potentially protect against atherosclerosis.

THE EFFECTS OF CIPROFIBRATE AND SIMVASTATIN TREATMENT ON CETP PLASMA AND MRNA LEVELS IN ADIPOSE TISSUE BIOPSIES IN MILDLY DYSLIPIDEMIC MEN

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Background: Cholesteryl ester transfer protein (CETP) plays an important role in HDL metabolism. The production of this plasma protein in liver and adipose tissue is in part controlled by cholesterol levels. **Objective:** To investigate how lipid lowering therapy affects CETP plasma levels, we determined the effects of ciprofibrate and simvastatin on CETP mRNA levels in adipose tissue biopsies and in an adipose tissue cell line (SW872). **Methods:** 7 men with mildly elevated LDLc (4.4±0.8 mmol/l) and 6 men with elevated triglycerides (4.9±2.2 mmol/l) underwent an abdominal adipose tissue biopsy before and after 2 months treatment with 40 mg simvastatin and 100 mg ciprofibrate, respectively. Poly-A+ mRNA was isolated from biopsies using MagNaPure. CETP and GAPDH mRNA levels were measured using a SybrGreen quantitative LightCycler PCR (Roche). CETP/GAPDH mRNA levels were expressed in arbitrary units. Plasma lipids were measured and plasma CETP concentrations were determined with ELISA. **Results:** A trend towards a correlation between plasma CETP levels and CETP mRNA in adipose tissue (r=0.391, p=0.072) was observed, but stepwise regression showed that only LDLc and not CETP mRNA levels contributed significantly to variations in plasma CETP levels. After treatment, TC, LDLc and TG were significantly reduced in the statin group, and TG was significantly reduced in the fibrate group. In the statin group, plasma CETP levels were reduced from 1.95±0.43 to 1.43±0.31 mg/l (p=0.018, paired t-test). In the fibrate group a smaller decrease was observed (1.65±0.39 to 1.41±0.40 mg/l, p=0.046). The decrease in CETP concentration was not accompanied by according changes in CETP mRNA levels in adipose tissue. Likewise, no changes in CETP mRNA levels were noted in SW872 cells after statin or fibrate treatment. Nevertheless, CETP mass changes could be explained by a regression model consisting of LDLc and CETP mRNA changes. Finally, CETP mRNA changes were highly correlated with TG changes (r=0.691, p=0.019). **Discussion:** These data suggest that both ciprofibrate and simvastatin may partly and indirectly control plasma CETP levels by affecting *CETP* gene transcription in adipose tissue via TG changes.

HS-CRP LEVELS ARE INCREASED IN CHILDREN WITH FH BUT NOT MODULATED BY PRAVASTATIN TREATMENT

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Inflammatory mechanisms play an important role in atherogenesis and its clinical sequelae. High sensitive C-reactive protein (hs-CRP) has emerged as a promising marker of inflammation in vascular disease. In this study, we examined the levels of hs-CRP in 8-18 years old children with familial hypercholesterolemia (FH) (n=207) as compared to healthy controls (n=84). We also evaluated the effect of 2-year pravastatin treatment on hs-CRP in FH children as compared to placebo.

Hs-CRP levels were significantly higher in FH children than in controls: 0.03 mg/dl (range 0.01, 2.12 mg/dl) vs 0.01 mg/dl (range 0.01, 1.08 mg/dl), respectively (*p*=0.017). Gender and BMI were identified as independent predictors of CRP. Although the relationship between hs-CRP, age and LDL-C reached significance in univariate analyses, this was lost in multivariate analyses. BMI was positively associated with hs-CRP and, unexpectedly, girls had higher hs-CRP levels than boys. Two-year treatment with 20-40 mg of pravastatin did not significantly reduce hs-CRP levels as compared to placebo treatment: the mean difference between pravastatin and placebo treatment was -0.016 mg/dl (95% CI: -0.115, 0.082 mg/dl).

Our findings show that hs-CRP levels are increased in children with FH, which suggests that inflammatory processes can already be elicited at a very young age. Although CRP levels were increased, they were still considerably lower than those in adults, which might explain why no effect was observed of pravastatin therapy, in contrast to adults.

REMOVAL OF LDL-C, PROCOAGULATORY MEDIATORS AND INFLAMMATORY BIOMARKERS BY H.E.L.P.-APHERESIS IN THE TREATMENT OF HEART TRANSPLANT ATHEROSCLEROSIS

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During the past decade, major improvement in our understanding of the mechanisms for the development of atherosclerotic lesions has emerged. Besides LDL cholesterol (LDL-C) inflammatory mechanisms seem to play a major role in the development of both spontaneous as well as transplant associated forms of the disease. In addition to elevated LDL-C and low HDL-C, increased circulating plasma levels of vascular inflammatory markers as well as prothrombotic factors may identify patients who are at risk for developing transplant atherosclerosis. LDL apheresis has proven beneficial in reducing cardiovascular events in patients who are refractory to drug therapy alone. H.E.L.P. therapy drastically reduces circulating levels of LDL-C, Lp(a) and procoagulatory mediators (Jaeger et al.). We recently showed that this therapy also significantly reduced the levels of soluble adhesion molecules (VCAM-1 and E-Selectin), monocyte chemoattractant protein 1 (MCP-1), inflammatory markers such as hs-CRP, endothelin, IL-6, LPS, LBP as well as prothrombotic factors sCD40L and tissue factor (Wang et al. 2004). We therefore initiated a study to investigate whether H.E.L.P. apheresis in combination with statins would be beneficial in prevention or arrest of heart transplant atherosclerosis. Ten years after heart transplantation the survival rate in patients (n=28) treated weekly with H.E.L.P. apheresis was 82 % as compared to 46 % in HTX-patients not undergoing this therapy. Thus this new therapy may not only delay but also prevent the development of graft vasculopathy and reduce the mortality after heart transplantation.

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CAROTID INTIMA-MEDIA THICKNESS (CIMT): TECHNICAL AND. CLINICAL VALIDATION: AN INDUSTRY PERSPECTIVE.

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Carotid ultrasound is a non-invasive, two-dimensional imaging modality that is used clinically to diagnose carotid artery stenosis in patients with findings consistent with stroke risk or a stroke/TIA event. For clinical research applications, carotid ultrasound has been used extensively to acquire one-dimensional measures of the carotid artery intima-media thickness. It is now recognized that increased CIMT, rather than carotid luminal narrowing, is a predictor of cardiovascular risk. This non-invasive measure of latent atherosclerosis has been used by the pharmaceutical industry as a measure of the disease progression and therapeutic efficacy provided by drugs under study. Ultrasound is a well-accepted technology for the diagnosis and treatment of vascular disease in clinical care. Its technical validation for such clinical practice applications is complete. The research use of ultrasound to measure CIMT and the rate of IMT thickening has been shown in large epidemiologic studies, to correlate with cardiovascular risk factors, as well as increased rates of coronary artery and cerebrovascular events. In a number of large clinical therapeutic trials assessing the efficacy of dyslipidemia and anti-hypertensive agents, the slowing of the progression of IMT thickening is consistently correlated with a reduction in clinical events. While there is some controversy regarding the imaging protocols to acquire and analyze measures of CIMT, there appears to be sufficient technical and clinical evidence supporting the use of carotid ultrasound as a surrogate marker of atherosclerosis progression and clinical outcomes in the evaluation of medical therapies.

CAROTID INTIMA-MEDIA THICKNESS (IMT) - METHODOLOGY, VALIDATION, AND UTILIZATION

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There have been multiple prospective epidemiological studies that have shown that increases in carotid artery IMT are associated with increased risk of incident cardiovascular events. The subsequent use of carotid IMT as the outcome measure in therapeutic trials compels improvement in the precision of the measurement and development of a standardized protocol. Most large epidemiology studies measure multiple sites in multiple segments of the carotid artery. Some measure both near and far wall, while others focus on just far wall. Some include the carotid bulb as part of the internal carotid artery (ICA), while others treat it as a separate segment. The variability of measurements made in the common carotid artery (CCA) is commonly found to be less than half that for the bulb and ICA. This argues in favor of focusing only on the CCA segment, particularly after the introduction of semi-automated edge detector techniques. However, IMT progression is slower in the CCA than the bulb and ICA. Furthermore, the pathology of the atherosclerotic plaque, which originates in the bulb and ICA, challenges the wisdom of focusing only on the straight segment of the carotid artery. Focal plaque, when included in measurement of IMT, will represent the site of maximum IMT thickening for a given subject. Furthermore, multiple studies demonstrated that different risk factors related to the separate segments of the carotid artery in different ways. Most investigators today chose to use a combined measure of the CCA and ICA as their primary IMT outcome measure. The strategies employed to reduce measurement error and increase power include averaging multiple measurements, combining measurements made from two separate scans taken in close temporal proximity, using automated edge detector techniques where possible, controlling for the cardiac cycle, and employing multiple angles of interrogation. Population based studies of IMT demonstrate an annual progression rate of 0.005mm/yr to 0.01mm/yr. Baseline IMT varies with age, sex, race and risk factors. For a healthy middle-age male, average carotid IMT will be approximately 0.6mm. Measurement error varies with wall thickness but is approximately 10% of baseline IMT in well-designed studies. This data determines the appropriate design for progression studies. It is clear that the longer the time interval of the trial, the larger the study population, the more comprehensive the imaging protocol, and the more significant the expected impact of the therapeutic intervention, the more likely divergent rates of IMT change will be identified. Meaningful differences in progression rates have be shown in trials involving one to two hundred subjects over a period of one to two years. The usefulness of carotid IMT change as an outcome measure in interventional trials, serving as a surrogate for clinical events, seems increasingly well established. There is universal recognition of the need for a standardized protocol to facilitate comparison of results among the many trials using this technique.

IMPACT OF CAROTID ULTRASOUND IMAGING STUDIES IN THE IDENTIFICATION AND PREVENTION OF ATHEROSCLEROSIS

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Carotid intima-media thickness (IMT) acquired by means of B-mode ultrasound imaging of carotid arterial walls has increasingly proven its value as an in-vivo, non-invasive vascular research tool. IMT can document arterial wall changes as a continuous variable throughout life in groups at cardiovascular disease risk and in unaffected controls. As supported by the results of epidemiological studies and drug trials, the method can investigate the need for vascular disease prevention and evaluate cardiovascular disease risk reduction by therapeutic regimens in populations at risk. Also, IMT data comply with the statistical definition of a surrogate biomarker. Consequently, IMT is considered a truly validated surrogate endpoint for atherosclerosis progression and future and present atherosclerotic disease risk. Presently, ultrasound arterial wall imaging studies go through a series of rapid methodological, technical and procedural developments. The approach to imaging studies is therefore standardization that allow for complementary observational and trial data that can be quality assessed and quality controlled. For those purposes, fully digitized state-of-the-art technology used in an environment of accessable imaging and image analysis, datamanagement and statistical analyses protocols is crucial. We address how and why this fascinating and elegant tool has widespread scientific and clinical applications in atherosclerosis research as well as its implications on cardiovascular disease prevention.

COULD IMT BE A VALUABLE SURROGATE FOR CLINICAL EVENTS IN NEW DRUG APPROVALS? A EUROPEAN PERSPECTIVE

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Estimation of the individual 10-year absolute cardiovascular risk is currently based either on counting the number of risk factors or on the calculation of scores such as the Framingham or PROCAM scores. These approaches are limited by their applicability to different populations with different level of cardiovascular risks. For example it has been estimated that FS overestimates the risk in UK population by a factor of 1.5 and in French population by a factor of 7.

Other approaches to estimate the risk of an individual exist. Intima media thickness of the common carotid artery represents a marker for subclinical atherosclerosis and an opportunity for an early detection of pre symptomatic individuals (i.e., the vulnerable patient). It has been associated with all modifiable (e.g., blood pressure, blood cholesterol, smoking, diabetes, obesity) and non modifiable risk factors (including age, gender, genes and currently unknown risk factors), with all ischemic stroke subtypes, with occurrence of future carotid plaque, and with a high risk of incident myocardial infarction, stroke and vascular death. Therapeutic interventions with blood pressure lowering agents, lipid lowering agents, as well as multifactorial interventions in diabetics, resulted in slowing progression or even reduction in carotid IMT. Carotid IMT has recently been recognized by the Food and Drug Administration as a surrogate for the evaluation of therapeutic intervention on atherosclerotic disease. However, the European Medicine Evaluation Agency has another point of view, mainly because it has not yet been proven that regression of clinical events parallel regression of carotid atherosclerosis.

Cross sectional and prospective epidemiologic studies of carotid atherosclerosis showed that increased IMT was the first change to appear before plaque occurrence. Plaque occurrence means a definite high absolute cardiovascular and stroke risk status. Therefore, increased IMT without plaque may represent the way to detect and target intermediate risk population on which prevention could be more efficient. A drug which significantly interferes with IMT progressions, plaque occurrence, or plaque progression is likely to prevent plaque vulnerability, and hence clinical events. Therefore, these ultrasound measures could be valuable outcomes for new drug approvals. Prospective interventional studies looking at these different stages of carotid atherosclerosis (CCA-IMT, plaque, plaque area) and clinical events are needed to explore such a hypothesis

THE ROLE OF VASCULAR IMAGING IN THE APPROVAL OF ANTIATHEROSCLEROSIS DRUGS - A REGULATORY PERSPECTIVE

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Clinical investigational strategies employing vascular imaging studies are increasingly required in cardiovascular drug development. Imaging of changes in vascular anatomy is likely to be necessary, if not sufficient, to support conclusions of efficacy of new drugs acting through novel (non-LDL-lowering) mechanisms.

There is no consensus on the best imaging methods for assessment of changes in vascular disease risk with treatments intended to alter atherosclerosis. Information on the comparative utility of different methods in assessing changes in anatomy that indicate changes in clinical risk is needed.

The division of metabolic and endocrine drugs requires sponsors to employ at least two different methods to image different vascular beds (e.g., carotid, coronary) in active-controlled studies of potential antiatherosclerosis drugs.

Studies of LDL-lowering agents have been "positive" using contrast angiography, carotid ultrasound, and more recently intravascular ultrasound These findings have been "validated" by the numerous large endpoint trials of statins and certain other agents) showing improvements in CV morbidity and mortality with active treatments that lower levels of atherogenic lipoproteins.

There are no data which address the minimum change in vascular anatomy by these methods that denotes a clinical benefit (reduction in CHD risk). The magnitude of change beyond that induced by known active agent that defines a further reduction in clinical risk requires definition. To date, we have not approved any new drugs based solely on these imaging "surrogates."

MEASUREMENT OF CAROTID ARTERY INTIMA-MEDIA THICKNESS IMPROVES THE POWER OF TRADITIONAL RISK FACTORS TO PREDICT CARDIOVASCULAR EVENTS.

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The intima media thickness (IMT) of the carotid artery plays a key role in atherosclerosis assessment. We performed a longitudinal observational study to investigate whether carotid Max-IMT, measured in clinical practice, can be combined with the Framingham Risk Score (FRS) to improve the prediction of cardiovascular events in patients at low to intermediate risk for such events. 1969 patients attending our Lipid Clinic received ultrasonic measurement of Max-IMT and its distribution was presented in age- and gender-specific tables from which a Max-IMT percentile (IMT_{perc}) was derived for each patient. 242 patients with low or intermediate risk (FRS <20%) were followed-up for about 5 years. Twenty four of the low/intermediate risk patients (FRS<20%) suffered a cardiovascular event in the next 5 years. Both FRS and IMT_{perc} were independent outcome predictors ($P<0.006$ and $p<0.02$, respectively; Cox model), with a hazard ratio of 9.5 (95% CI 2.4, 37.5; P=0.001) in patients in whom both IMT_{perc} and FRS were above specified values. In Kaplan-Meier analyses, adding the IMT_{perc} improved the predictive value of the FRS ($\chi 2=13.7$, p=0.003; log-rank test). Patients with elevated values (even though FRS<20%) had the same risk as patients with 20%<FRS<30%. The FRS underestimated the risk for patients with IMT_{perc} above the specified value. An equation to adjust the FRS on the basis of a raised IMT_{perc} is suggested. The combined use of conventional risk factors and ultrasonic measurements of carotid IMT significantly increases the ability to predict cardiovascular events in low or intermediate risk patients.

PHARMACOGENETICS OF STATINS AND FIBRATES

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Statins can normalize the entire lipoprotein spectrum except HDL in CHD patients, and also lower CRP. The combination of a statin and a CETP inhibitor will more than normalize the entire profile, but its use awaits outcome trials. The statins inhibit cholesterol biosynthesis, plasma lathosterol, and can decrease the production of apoB-100 containing lipoproteins, as well as enhancing their clearance. There is variability among patients in terms of LDL cholesterol lowering in response to statins. Statins can increase intestinal cholesterol absorption and plasma beta carotene levels. Benefit in CHD is associated with decreases in plasma lathosterol and lack thereof with increases in plasma beta sitosterol in subjects on the statin/niacin combination. Genetic variation at the apoE and ATP binding cassette G5 and G8 gene loci can affect cholesterol absorption and LDL cholesterol lowering response to statins. Patients with enhanced cholesterol absorption on statins appear to be less responsive, and may be those who would get the greatest benefit from the addition of ezetimibe. Genetic variation at the HMG CoA reductase gene locus has also been shown to affect statin responsiveness. Fibrates are the second most widely used class of lipid lowering agents, and are very effective in lowering triglycerides (TG), remnant lipoproteins, and CRP, altering lipoprotein subspecies, and they can substantially lower CHD risk especially in subjects with elevated insulin levels There is a great deal of variability in TG lowering response to the same dose of the same fibrate. Genetic variation at the lipoprotein lipase and PPAR alpha and gamma gene loci affects this response to fibrates as well as outcomes in the VA HIT in our own analyses. The statin-fenofibrate combination is well tolerated and should be especially effective in high risk subjects with elevated insulin levels, who may not derive that much benefit from a statin alone.

THE CLEARANCE OF STATINS AND ITS RELEVANCE IN DRUG-DRUG INTERACTION

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The liver biotransforms all statins, which accounts for their overall low systemic bioavailability. The apparent total body clearance is very high because of an important hepatic first-pass effect. With the exception of pravastatin, which is transformed enzymatically in the liver cytosol, all statins undergo extensive microsomal metabolism by the cytochrome P450 (CYP) isoenzyme systems. The CYP3A4 isoenzyme is responsible for the metabolism of lovastatin, simvastatin, and atorvastatin. Fluvastatin is metabolized primarily by the CYP2C9 and rosuvastatin, not extensively metabolized, has some interaction with the CYP2C9 enzyme. The amount of the dose of statin that is excreted in urine varies from negligible amounts for atorvastatina to 10 and 20%, respectively, for rosuvastatina and pravastatin. In particular, pravastatin and rosuvastatin differ from other statins in that they show a dual route of elimination. This is particular true when the drugs are given intravenously; after this route of administration, the renal excretion of pravastatina attains 47% of the administered dose, corresponding to a renal clearance of about 450 mL/min and 28% for rosuvastatina (Cl_r=225 ml/min). Tubular secretion is a predominant mechanism in the renal excretion of pravastatin and rosuvastatin . All other statins and their metabolites are excreted mainly via the bile into feces. A newly recognized class of active drug transporters, including the P-glycoproteins, is known to affect the disposition and bioavailability of many drugs, including CYP3A4 substrates. Transport proteins are, at least in part, responsible for the low and variable oral bioavailability of atorvastatin, lovastatin, simvastatin, and pravastatin. Indeed, interactions with other drugs at the P glycoprotein level could potentially be responsible for the rhabdomyolysis observed after statin– –digoxin combination therapy. Rosuvastatin, and fluvastatin have been also shown to be recognized by these transporters. These pharmacokinetic differences can affect the potential for drug interactions with statins, which can result in markedly increased or decreased plasma concentrations of some drugs within this class. Concomitant use of certain drugs (genfibrozil, erythromycin, itraconazole, and immunosuppressive drugs such as cyclosporine) can increase blood levels of statins and, consequently, the risk for myopathy.

ROSUVASTATIN PROVIDES ANTI-INFLAMMATORY EFFECTS AND END-ORGAN PROTECTION IN STROKE-PRONE RATS

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Spontaneously hypertensive stroke-prone rats (SHRSP) develop brain abnormalities, preceded by systemic inflammation characterized by the accumulation, in the serum and urine, of several acute-phase proteins. Thus, this experimental model represents a useful tool to evaluate inflammation in the context of brain injury. We investigated whether rosuvastatin influences the development of inflammation associated with brain abnormalities in salt-loaded SHRSP. SHRSP, fed a high salt-diet, were treated long-term with vehicle or rosuvastatin (1 and 10 mg/kg/day). Brain abnormalities developed after 40±5 days and after 60±5 days ($p<0.05$) of salt-loading, in the vehicle and in rosuvastatin-treated (1 mg/kg/day) SHRSP respectively. After 100 days of treatment, no damage was detectable in 30% of the rats treated with the highest dose of drug. Rosuvastatin treatment attenuated the expression of MCP-1, TGF-β1, IL-1β and TNF-α in the kidney, and of P-selectin in brain vessels.

Furthermore, rosuvastatin treatment increased the mRNA expression of eNOS in the aorta, as compared with vehicle-treated SHRSP. Urinary excretion of acute-phase proteins increased in vehicle-treated but remained negligible in the drug-treated animals. These effects are independent of changes in cholesterol levels or other physiological parameters such as blood pressure. Treatment of SHRSP with simvastatin (2-20 mg/kg/day) did not exert any protective effects in this animal model. Our observations support the hypothesis that rosuvastatin represents a novel means of attenuating inflammatory processes associated with cerebrovascular disease.

PPAR AND LXR ACTIVATION MODULATES THE EXPRESSION OF KEY-GENES INVOLVED IN REVERSE STEROL TRANSPORT AT THE BLOOD-BRAIN BARRIER

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Liver X receptors (LXRs) and peroxisome-proliferator activated receptors (PPARs) control the transcription of key-genes involved in peripheral reverse cholesterol (C) transport. The brain covers a major part of its C demand by de novo synthesis and C output is partly accounted for by the formation and secretion of 24*(S)*OH-C across the blood-brain barrier (BBB; Björkhem et.al. J Biol Chem 2001;276:37004). We reported that ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor, class B, type I (SR-BI) play major roles as modulators of sterol mobilization at an *in vitro* BBB constituted of primary porcine brain capillary endothelial cells (pBCECs; Panzenboeck et.al. J Biol Chem 2002;277:42781). SR-BI at the apical membrane supports the efflux of 24*(S)*OH-C across the BBB to plasma HDL acceptors. In addition, treatment with the LXR agonist 24*(S)*OH-C enhances C transport via the ABCA1/apoA-I-dependent pathway. Here we report on the effects of synthetic LXR- (TO-901317) and PPAR- (thiazolidinediones and fibrates) agonists on polarized expression of ABCA1, SR-BI, and apoA-I and on sterol efflux from pBCECs. Despite a significant up-regulation of predominantly apical membrane SR-BI protein by PPAR agonists, efficient HDL dependent mobilization of 24*(S)*OH-C from (polarized) pBCECs was unaffected. By contrast, TO-901317 and (certain) PPAR agonists increased apoA-I-mediated C efflux from pBCEC monolayers up to 2-fold. Up-regulation of ABCA1 protein expression by LXR and PPAR agonists was reflected by enhanced apoA-I dependent C efflux from polarized pBCECs. This, together with an increased secretion of apoA-I observed upon treatment with PPAR agonists may contribute to the formation of HDL-like particles at the BBB. We postulate that PPARs and LXRs may provide promising (drug) targets for modulating sterol metabolism at the BBB.

HIGH DOSE STATIN TREATMENT AFFECTS SKELETAL MUSCLE STEROL AND UBIQUINONE METABOLISM IN MAN: A RANDOMISED, CONTROLLED TRIAL

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It is not know whether currently used high doses of statins are able to function in skeletal muscles. This study was designed to assess effect of high dose statin treatment on cholesterol and ubiquinone metabolism and mitochondrial function in human skeletal muscle.
Forty-eight hypercholesterolemic patients (33 men and 15 women), were randomly assigned to receive 80 mg/d of simvastatin (n=16), 40 mg/d of atorvastatin (n=16), or placebo (n=16) for eight weeks. Plasma samples and muscle biopsies were obtained before and at the end of the follow-up.
Both statins resulted in similar reductions in circulating ubiquinone, cholesterol, triglyceride and lipoprotein levels. Plasma plant sterol concentrations increased only in the atorvastatin group. Simvastatin increased muscle cholesterol levels by 38% and muscle plant sterol levels were significantly increased with both statins. Muscle ubiquinone concentrations were significantly reduced by 30% in the simvastatin group. Respiratory chain enzyme activities will be presented in the meeting.
High dose statin treatment lead to significant changes in the skeletal muscle sterol and ubiquinone metabolism. In particular, muscle plant sterol levels were significantly increased during statin treatment.

REDUCTION OF SERUM LEVEL OF VEGF IN HYPERCHOLESTEROLEMIC PATIENTS UNDER SIMVASTATIN TREATMENT

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Vascular endothelial growth factor (VEGF) has been proposed to play an important role in the pathogenesis and progression of atherosclerotic plaques by different pathways. Interleukine (IL-) 6, a cytokine increased in acute and chronic inflammatory conditions, can induce the production of VEGF in vascular smooth muscle cells (SMC). The suspicion has been raised that anti-inflammatory effects of statins contribute to the clinical benefits of these drugs. In this study, we investigate the influence of statin treatment on the serum level of VEGF in hypercholesterolemic patients. 107 hypercholesterolemic patients were treated with 20 (n=52) or 40mg (n=55) of simvastatin daily. 6 weeks treatment with simvastatin resulted in a significant reduction of VEGF ($p<0.001$) in serum of patients. Corresponding effects have also been observed after 6 months therapy. With respect to SMC, IL-6 induced the expression of VEGF in human umbilical vein derived SMC as analyzed by rt-PCR and flow cytometry. Co-incubation of IL-6 stimulated SMC with atorvastatin, cerivastatin or simvastatin led to a significantly decreased expression of VEGF mRNA as well as proteins by these cells. Based on our results we hypothesize, that these anti-inflammatory effects of statins might support the clinical benefits of these drugs.

PLASMA PLTP ACTIVITY IS DECREASED BY ATORVASTATIN TREATMENT OF TYPE 2 DIABETES MELLITUS PATIENTS

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Background Phospholipid transfer protein (PLTP) plays an important role in lipoprotein metabolism, but effects of statins on PLTP are unknown. Earlier studies showed that PLTP activity is elevated in type 2 Diabetes Mellitus (DM) patients with the atherogenic lipoprotein phenotype, e.g. low HDL-C and elevated triglycerides (TG). Case-control studies have suggested that plasma PLTP activity may be a risk factor for coronary artery disease.
Methods We measured PLTP activity and PLTP mass in 108 patients with DM and the atherogenic lipoprotein profile as a substudy of the DALI study, a 30-weeks randomised, double blind, placebo-controlled trial with Atorvastatin 10 mg and 80 mg. PLTP was measured using published methods: PLTP activity with the liposome vesicles-HDL system and PLTP mass with a sandwich ELISA.
Results Atorvastatin treatment resulted in decreased PLTP activity (A10: -9%, $P<0.05$; A 80: -13%, $P<0.002$), whereas plasma PLTP mass showed a tendency to increase (A10: 5%; A80: 11%), leading to a substantial decrease in mass-adjusted activity. No increase in plasma HDL-C was observed, despite a 25-35% decrease in plasma TG. LDL-C decreased by 41-52%. The decrease in PLTP activity was positively correlated with a decrease in the atherogenic parameters total cholesterol, RLP-C, apoB, apoCIII and CETP mass (all $P<0.05$).
Analysis of baseline data showed the following correlations. PLTP mass was positively correlated with HDL-C and apoAI ($P<0.001$) and negatively with TG, LpB-CIII, CETP mass and RLP-C (all $P<0.005$). PLTP activity was positively correlated with waist-hip ratio, HbA1C, and plasma glucose ($P<0.05$). As in most studies, PLTP mass and activity were only weakly correlated ($r=0.25$, $P<0.05$). PLTP data were analysed by quartiles. The highest quartile of PLTP mass (11.4 ± 1.9 mg/L) was found in patients with plasma TG 2.5 ± 0.9 mmol/L and HDL-C 1.21 ± 0.22 mmol/L. Patients with PLTP mass in the lowest quartile (4.9 ± 1.0 mg/L) had much higher plasma TG (3.7 ± 1.3 mmol/L) and very low HDL-C (0.9 ± 0.1 mmol/l).
Conclusion The potentially atherogenic activity of PLTP in type 2 DM is decreased by treatment with Atorvastatin. Low PLTP mass is an indicator for the atherogenic lipoprotein profile.

APOLIPOPROTEIN A-II PLASMA LEVEL AND HDL CHOLESTEROL-ACCEPTING CAPACITY IN MEN WITH HYPERLIPIDEMIA

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Apolipoprotein A-II (apo A-II) is the second most abundant protein of human HDL but its function remained unclear. This study aimed to find out if there is a relationship between apo A-II plasma concentration and cholesterol-accepting and cholesterol-transporting function of HDL in men with hyperlipidemia. Forty-two men aged 50-69 with total cholesterol (C) level 190 mg/dl and/or triglycerides (TG) 150 mg/dl were included into the study. Apo A-II concentration was determined by immunoturbidimetric assay. Sera efflux potential was measured after their incubating with [^{3}H]cholesterol-labeled Fu5AH hepatoma cells. The results demonstrate a relationship between apo A-II level and C: R=0,6185, p<0,001, TG: R=0,3695, p=0,016, apo B: R=0,5257, p<0,001 and HDL phospholipids (HDL-PL): R=0,5786, p<0,001. All patients were subdivided into tertiles according to their apo A-II level which comprised (M±SD, mg/dl) 30,8±3,3, 36,5±1,0 and 43,9±2,9, respectively. No significant differences in plasma HDL-C and apo A-I levels between tertiles were found. Patients from the 3rd tertile exhibited higher C, TG, LDL-C and apo B levels as compared to other tertiles. The HDL-PL concentration in the 3rd tertile appeared to be the highest without any changes in PL composition and was accompanied by significant increase in HDL cholesterol-accepting ability: cholesterol efflux was 16,2±3,9% vs 13,1±1,6% and 15,7±1,6%, respectively. Thus, elevated apo AII level was associated with atherogenic combined hyperlipidemia but was not related to the efficiency of HDL-mediated reverse cholesterol transport from peripheral tissues to the liver. However, the cholesterol-accepting properties of HDL in patients with high apo AII level were enhanced.

PHYSICO-CHEMICAL AND BIOLOGICAL FUNDAMENTALS FOR PHENOTYPING (TYPING) HYPERLIPOPROTEINEMIAS

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All lipoprotein (LP) fractions involved in the phenotyping of hyperproteinemia (HLP) are formed by a single lipid-transporting protein: apoB. By binding different amounts of nonpolar lipids, first triglycerides (TG) and later cholesterol esters, in enterocytes apoB-48 forms chylomicrons (Chy), in hepatocytes apoB-100 forms very low density LP (VLDL), and low density lipoproteins (LDL) are formed in the blood. After part of TG is hydrolyzed, apoB conformation and surface charge change in all the LP. Preligand Chy and VLDL are secreted in the blood by enterocytes and hepatocytes, respectively, and preligand LDL are formed from VLDL. Ligand-Chy, VLDL and LDL, which are uptaken by cells, appear in the blood as a result of lipolysis. If cells with receptor defects cannot uptake ligand Chy, VLDL and LDL, further hydrolysis of TG leads to the formation of postligand-Chy, VLDL and LDL which either cannot be uptaken by cells. Accumulation of preligand-Chy in the blood underlies type I HLP. Postligand LDL lay the basis of type IIa HLP. Preligand-LDL form type IIb HLP. Together, postligand-Chy and postligand VLDL cause type III HLP. Preligand-VLDL form type IV HLP. Type V HLP results from simultaneous accumulation of preligand Chy, VLDL and LDL. Each phenotype of HLP is formed by LP with individual apoB conformation and nonpolar lipid ratio.

THE COMPOSITION, STRUCTURE AND THE BINDING OF VLDL AND LDL TO THE LDL RECEPTOR AT NORMO- AND HYPERTRIGLYCERIDEMIA: RELATION TO APOE PHENOTYPE

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The composition, apolipoprotein structure and lipoprotein binding to the LDL receptor were studied for VLDL and LDL particles isolated from subjects with apolipoprotein E phenotype E3/3 (E3), E2/2 or E2/3 (E2+) and E3/4 or E4/4 (E4+) in a wide range of plasma TG content. The data combined for all three phenotype groups were as follow. (1) The decrease of accessibility of VLDL tryptophan residues to I^- anions with the increase of VLDL dimensions originated from protein-protein interactions increasing with plasma TG. (2) The monotonous increase of quenching constant of LDL apoB fluorescence with TG/cholesterol (Chol) ratio reflected the "freezing" effect of Chol molecules on apoB dynamics. (3) ApoE-mediated VLDL binding and apoB-mediated LDL binding to the LDL receptor in a solid-phase binding assay proceeded by different mechanisms, being the same for particular lipoprotein from E3/3 or E2/3 subjects. (4) The "spacing" effect of apoC-III molecules on apoE-mediated VLDL binding resulted in the decrease of the number of binding sites. (5) A dependence of the affinity constant for LDL binding on tryptophan relative density passed through a maximum that corresponded to LDL intermediate size. For separate groups, VLDL particles from hypertriglyceridemic E2/3 heterozygotes possessed remnant-like properties (increased Chol, apoE and decreased apoC-III content) while binding efficiency was normal. Based on affinity constant values and LDL-Chol content, a competition between VLDL and LDL for the binding to the LDL receptor, that increases with plasma TG, is suggested, LDL from hypertriglyceridemic E3/3 homozygotes being the most efficient competitor.

THE STRUCTURE OF THREE RECOMBINANT APOLIPOPROTEIN E ISOFORMS IN SOLUTION

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Three apoE isoforms produced by heterologous expression in E. coli had similar global organizations consisting of two structural domains of different stabilities as shown by limited proteolysis and denaturation with guanidine hydrochloride. They were all highly alpha-helical and multimeric in solution. Sequential denaturation with guanidine hydrochloride led first to the formation of a monomeric intermediate, composed of a fully denatured carboxyl-terminal domain and a fully folded amino-terminal domain followed by the unfolding of the amino-terminal domain with generation of completely unfolded monomer. The non-coincidence of the denaturation process as followed by three techniques suggests that the unfolding of the amino-terminal domain is more complex than a simple two state process and suggests the presence of an intermediate. A three state treatment of the denaturation curves revealed the order of stability both for a whole protein molecule and the amino-terminal domain as apoE2>apoE3>apoE4, whereas the carboxyl-terminal domains had roughly similar stabilities. However, the stability of a given domain was different depending upon the technique used to follow the denaturation. Although all three isoforms possess a similar domain structure, there are isoform-specific differences in the stability and in the state of association in aqueous solution and the unfolding of the amino-terminal domain may be more complex than a simple two-state transition. The plasma apoE with intermediate "molten globule" state critical in lipid binding (Dergunov et al. Spectrochim Acta part A 2003;59:1127-37) possesses a similar two-domain structure.

INFLUENCE OF NEWLY SYNTHESIZED CHOLESTEROL ON BILE ACID SYNTHESIS IN HUMANS

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Hepatic bile acid synthesis is a key step in the maintenance of cholesterol homeostasis. The role of newly synthesized cholesterol on the biosynthetic pathway and on the limiting enzyme, cholesterol 7α-hydroxylase, is controversial. AIM of this study was to investigate the effect of simvastatin, a competitive inhibitor of HMG-CoA reductase, on cholesterol and bile acid synthesis in humans, during chronic pharmacological interruption of the enterohepatic circulation. PATIENTS AND METHODS. We investigated six patients with hypercholesterolemia in basal conditions, after 6-8 wk treatment with the bile acid-binding resin cholestyramine (8-16 g/day) and after further 6-8 wk with addition of simvastatin 40 mg/day. *In vivo* 7α-hydroxylation rate, a measure of bile acid synthesis, was determined by isotope release. Serum lathosterol levels, a marker of cholesterol synthesis, were determined by GC-MS. RESULTS. Plasma total and LDL-cholesterol levels significantly decreased (by 26% and 30% respectively) during cholestyramine alone and further decreased (by 47% and 55%) after adding simvastatin. Bile acid synthesis increased nearly 4-fold with cholestyramine; addition of simvastatin induced a significant decrease of 7α-hydroxylation rates (cholestyramine alone, 1591±183 mg/day; plus simvastatin, 1098±232, mean±SEM; $p < 0.01$, paired *t*-test). Serum lathosterol/cholesterol ratio and 7α-hydroxylation rates were significantly correlated ($r = 0.79$, $p < 0.01$). CONCLUSIONS. During chronic stimulation, treatment with statins induces a relative reduction of both cholesterol synthesis and bile acid synthesis rates. Newly synthesized cholesterol availability may be a limiting factor for bile acid synthesis in stimulated conditions, suggesting a feedforward effect which might involve coactivators of cholesterol 7α-hydroxylase transcription.
GRANT SUPPORT. Supported by COFIN grant MM06175714.

REPLACING CHOLESTEROL WITH DESMOSTEROL IN THE MOUSE – MARKEDLY INCREASED STEROL SYNTHESIS WITH UNCHANGED NET PRODUCTION OF BILE ACID

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Cholesterol homeostasis was studied in mice with a genetic inactivation of the 3 beta-hydroxysteroid-delta 24-reductase causing replacement of most of the cholesterol with desmosterol and reduced sterol levels in tissues and circulation.
There was a compensatory increase in hepatic sterol synthesis as shown by increased levels of hepatic HMG CoA reductase mRNA and markedly increased excretion of neutral sterols in faeces. Hepatic mRNA levels of HMG CoA synthase, SREBP-1, ABCG1, ABCG8 and ABCG5 were also increased. As judged from the fecal excretion total formation of bile acids was similar in knock out mice and in controls. The composition of bile acids was changed with reduced formation of cholic acid, the most effective suppressor of Cyp7A1 in mice. It was shown that both Cyp7A1 and Cyp27 are active towards desmosterol. Relatively high levels of 27-hydroxylated desmosterol were found in the circulation of the knockout mouse, demonstrating that the Cyp27-mediated degradative pathway to bile acids was active. In contrast to the hepatic changes, only modest changes in gene expression was observed in the brain, with a slight reduction in levels of mRNA corresponding to HMG CoA reductase.
The results show that the accumulation of desmosterol and the sterol depletion is associated with an upregulation of hepatic enzymes involved in both synthesis and metabolism of cholesterol. Desmosterol appears to be degraded to bile acids by both the neutral (Cyp7A1-mediated) and the acid (Cyp27-mediated) pathway. In contrast to the liver, very modest changes in gene expression were observed in the brain.

CHOLESTEROL IS THE MAJOR COMPONENT OF PLASMA LIPOPROTEINS ACTIVATING THE P38 MAPK.

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We have recently shown that LDL induced activation of the p38 MAPK pathway in fibroblasts. This resulted in cell spreading and lamellipodia formation, processes that may participate in wound healing or during vessel wall remodeling as in atherosclerosis. Our aim here was to determine which LDL components mediate the activation of the p38 MAPK pathway.

Testing the ability of LDL and HDL to activate p38 MAPKs demonstrated that both lipoproteins are able to induce p38 activation. Expressing the p38 response as a function of the concentration of the various lipoprotein components showed that LDL and HDL had the same potency in inducing p38 MAPKs when cholesterol, but not the other components, was used to express their concentration. This indicates that cholesterol is responsible for the activation of the p38 MAPKs by lipoproteins. Consistent with this notion are our observations that:

1) LDL derived lipids containing cholesterol and phospholipids were able to activate p38 MAPKs.
2) Phosphatidylcholine and sphingomyelin, the two main lipoprotein phospholipids did not induce p38 activation.
3) Cholesterol solubilized in β-methyl-cyclodextrin activated p38 MAPKs.
4) Vesicles made of phosphatidylcholine and cholesterol, but not vesicles made of phosphatidylcholine only, potently activated the p38 MAPK pathway.
5) Native LDL more strongly activated the p38 MAPKs compared to LDL with lower cholesterol content.

Our results suggest that elevated cholesterol content in LDL, as seen in hypercholesterolemia, favors the activation of stress pathways such as the p38 MAPK pathway, and therefore could contribute to atherosclerosis.

PCSK9 MUTATIONS FOUND IN PATIENTS DIAGNOSED WITH AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA IN THE NETHERLANDS

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Introduction: Autosomal dominant Hypercholesterolemia (ADH) has been identified as a major risk factor for coronary artery disease (CAD) and is associated with mutations in the LDL-receptor and apoB genes. Mutations in these two genes explain only 77% of our ADH patients. Recently, proprotein convertase subtilisin/kexin 9 (PCSK9) has been identified as a third locus responsible for ADH. **Patients and methods:** In 300 patients with non-LDL-receptor-, and non-apoB-linked hypercholesterolemia the complete coding sequence and intronic boundaries of PCSK9 was sequenced. **Results:** We identified four (1.3%) different amino acid substitutions, not found in 400 control individuals (Table).

Table: putative functional genetic variants found in the PCSK9 gene.

exon	variant	conserved AA	carriers	carriers unaff.
2	P71L	no	6	3
3	R160Q	yes	5	5
5	A220T	yes	1	0
9	S465L	yes	10	2

Family investigation did not show complete co-segregation of the mutations with the ADH phenotype: 10 of 22 carriers (Table) did not have LDL-cholesterol levels above the 95th percentile (disease penetrance of 0.55). **Conclusion:** The results of our study indicate that there is reasonable doubt that mutations in PCSK9 cause the ADH phenotype. Further investigation of the effect of mutations on PCSK9 activity and its regulation on molecular level is essential to obtain more insight in the involvement of PCSK9 in lipoprotein metabolism.

THE IDENTIFICATION OF NOVEL APOB MUTATIONS CAUSING FAMILIAL HYPOBETALIPOPROTEINEMIA.

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Introduction: Familial hypobetalipoproteinemia (FHBL) is caused by mutations in the apoB gene and characterized by low levels of LDL-C and apoB. These reductions in LDL-C and apoB have been suggested to protect against atherosclerosis. The prevalence of FHBL due to apoB gene mutations is estimated between 1/200 and 1/75. **Methods:** We identified 27 FHBL index cases from Dutch and Spanish decent, with plasma LDL-C and apoB levels below the 5th percentile. To identify the cause of the FHBL phenotype, we analyzed the promoter and complete coding region of the apoB gene by direct sequencing. **Results:** In 11 out of 27 probands an apoB gene mutation was found, associated with a truncated form of apoB and co-segregating completely with the FHBL phenotype. In four cases known mutations (R412X and 11712delC) and in 7 cases novel truncated apoB gene mutations, two frame-shift (1718delAT and 2534delA) and two nonsense mutations (Q1309X and R2507X), were found. Although an inherited FHBL trait was seen, in 16 cases no causal apoB gene mutations could be identified. **Conclusion:** 11 out 27 index cases were carrier of a proven truncated apoB mutation, indicating a much higher prevalence of FHBL in our study group. Since FHBL subjects may be protected against atherosclerosis, linkage analysis in the remaining families could direct us to other genes responsible for a FHBL phenotype and provide more insight in the mechanisms influencing apoB metabolism.

INFLUENCE OF APOLIPOPROTEIN E POLYMORPHISMS ON SERUM URIC ACID AND CREATININE LEVELS AS WELL AS ON PREDICTED GLOMERULAR FILTRATION RATE IN HEALTHY SUBJECTS

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Background: There are conflicting results regarding the effect of apolipoprotein E polymorphisms on the progression of a variety of renal diseases. However, there are no data on the possible effect of the apolipoprotein E alleles on serum creatinine levels, predicted gromerular filtration rate (GFR), as well as on serum uric acid levels in healthy subjects.

Methods: Two hundred-ninety apparently healthy individuals were studied. Apolipoprotein E genotyping was performed by the polymerase chain reaction, while prediction of the GFR was made by the Modification of Diet in Renal Disease equation (MDRD).

Results: Apolipoprotein (Apo) E2 was associated with lower levels of total cholesterol, LDL cholesterol and non HDL cholesterol, as well as with higher levels of triglycerides. Furthermore, ApoE2 allele was associated with increased serum creatinine levels compared to both the E3 and E4 alleles (1.04±0.13 vs 0.92±0.13 vs 0.88±0.11 mg/dl, respectively, p=0.0077), while the MDRD-predicted GFR was decreased in ApoE2 carriers compared to both E3 and E4 carriers (80.3±10.2 vs 88.1±9.6 vs 89.3±9.7 ml/min/1.73m^2, respectively, p=0.031). These observations remained statistically significant even if the effect of ApoE polymorphisms on age- and BMI-adjusted serum creatinine and MDRD-predicted GFR was separately analyzed in both men and women. Although, ApoE4 carriers tended to exhibit lower levels of serum creatinine and higher values of predicted GFR compared to the E3 carries, these differences did not reach statistical significance. Finally, ApoE2 allele was associated with higher serum urate levels compared to both the ApoE3 and ApoE4 alleles (5.4±2.1 vs 4.5±1.6 vs 4.0±1.0 mg/dl, respectively, p=0.01).

Conclusions: Apolipoprotein E2 allele seems to be associated with increased serum creatinine and urate levels, as well as with decreased MDRD-predicted GFR in healthy subjects.

LIPOPROTEIN KINETICS AS MEASURED WITH STABLE ISOTOPES IN FAMILIAL COMBINED HYPERLIPIDEMIA AND IMPAIRED GLUCOSE TOLERANCE

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Increases in triglyceride (TG) levels in the plasma have been described both in patients with impaired glucose tolerance (IGT) or Type 2 Diabetes (T2D) and in persons from families with familial combined hyperlipidemia (FCHL). The knowledge of the underlying pathomechanisms of the TG increases is of diagnostic and therapeutic relevance. We aimed at measuring the in vivo lipoprotein turnover in the mentioned groups of patients (IGT: n = 5; T2D: n = 5; FCHL: n = 5) in comparison with metabolically healthy controls (n = 5). The Body Mass Index was similar in the groups. Triglyceride concentrations in IGT probands were slightly elevated, while they were clearly elevated in T2D and FCHL. Lipoprotein lipase and hepatic lipase were similar in these groups. In vivo labelling of apolipoprotein B was done with a bolus injection and a constant infusion for 12 hours of L- [ring-13C6] phenylalanine or L- [5,5,5-2H3] leucine. The enrichment of isotopes and ratio of tracer/tracee was measured by GC/MS. Both the IGT and the T2D groups had an increased VLDL 1 pool due to an enhanced hepatic VLDL 1 synthesis. The fractional catabolic rates were not different between the groups. In contrast, the VLDL 2 pool was increased in IGT and FCHL probands. In IGT probands only the input out of VLDL 1 into VLDL 2 was clearly increased. On the other hand, only in FCHL probands the direct hepatic synthesis of VLDL 2 was increased. In addition, we measured mevalonic acid and lathosterol in the plasma. Both parameters were clearly elevated in FCHL probands only and correlated with VLDL 2 synthesis rate in these probands. In conclusion, it appears that in probands with IGT or T2D on one hand and those with FCHL the VLDL production is different and differently regulated.

SECRETED TYPE II PHOSPHOLIPASE A2 DECREASE HDL-PHOSPHOLIPIDS AND HDL3-CHOLESTEROL IN HUMAN

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Backgrounds: Atherosclerosis is recognized as a chronic inflammation of vessel walls. Plasma levels of secreted type II phospholipase A2 (sPLA2) significantly increase during acute and chronic inflammatory states. sPLA2 is highly expressed in human atherosclerotic arterial walls. Increased levels of sPLA2 in mice were associated with decreased levels of HDL-cholesterol. Thus, sPLA2 may be a key molecule, which acts between atherogenesis and lipid metabolism.
Methods: We enrolled consecutive 92 male subjects (mean age 60 yr.) without taking any lipid lowering agents who underwent elective coronary angiography.
Results: Mean plasma sPLA2 levels with or without coronary artery disease (CAD) were not different significantly. Regression analysis showed significant correlation between plasma sPLA2 levels and serum high sensitive CRP levels ($r=0.42$, $p<0.01$). We found significant negative correlation between plasma sPLA2 levels and serum apolipoprotein A-I ($r=-0.23$, $p=0.03$), A-II ($r=-0.29$, $p=0.006$), HDL-phospholipids (PL) ($r=-0.24$, $p=0.02$), and HDL3-cholesterol ($r=-0.32$, $p=0.003$). In summary, (1) plasma sPLA2 levels were highly associated with inflammatory marker (high sensitive CRP); (2) increased levels of plasma sPLA2 were associated with decreased levels of serum apolipoprotein A-I, A-II, HDL-PL, and HDL3-cholesterol.
Conclusion: We conclude that sPLA2 play a role in the catabolism of HDL-PL and associated with lower HDL3-cholesterol levels in human.

APOLIPOPROTEIN A-V POLYMORPHISMS AND REMNANT-LIKE PARTICLE CONCENTRATION

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It has been repeatedly shown that T-1131C and Ser19Trp polymorphisms in apolipoprotein A-V gene (*APOAV*) are associated with increased level of plasma triglycerides (TG). However, the mechanisms behind this effect are not understood yet. Therefore, the hypothesis that these *APOAV* variants affect catabolic pathways that could result in accumulation of remnants was tested. The concentration of remnant-like particle-cholesterol and triglyceride (RLP-C and RLP-TG, respectively) was measured after immunoprecipitation of the other lipoproteins with monoclonal antibodies against apo B-100 and apo A-I in 1% population sample from one district of the Czech republic; the relationship between the RLP-C and RLP-TG concentrations and three *APOAV* variants (T-1131C, Ser19Trp, and Val153Met) was then analyzed. The population sample consisted of 131 men and 184 women (age: 55±12 and 56±11 years; cholesterol: 5.4±1.1 and 5.6±1.2 mmol/l; TG: 2.2±1.8 and 1.9±1.2 mmol/l; RLP-C: 0.32±0.54 and 0.25±0.31 mmol/l; RLP-TG: 0.45±1.04 and 0.21±0.40 mmol/l, respectively). None of the three studied *APOAV* variants had any effect on concentration of RLP-C and/or RLP-TG in the whole sample and in both men and women, when analyzed separately. In conclusion, our data do not support the idea that the variation in apolipoprotein A-V gene has any significant impact on the catabolism of triglyceride-rich lipoproteins as determined by measurement of RLP-C and RLP-TG concentration.

Supported by grants No. NJ-7600-2 and No. NR-7923-3 from IGA MH CR.

INTERACTION BETWEEN HUMAN LDL AND PROTEOGLYCANS, STUDIED IN A SOLID PHASE SYSTEM

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The presence of small dense LDL increases a person's risk of cardiovascular disease. Hypertriglyceridemic persons often have high levels of small dense LDL. The suggested mechanism for the atherogenicity of these particles is that they have a higher affinity for proteoglycans. The aim of this study was to investigate the binding of LDL particles of different composition to decorin in a solid phase system. LDL from individuals with normal (nTG) (n=10) and high (hTG) (n=10) S-triglyceride levels was isolated using deuterium oxide. Decorin was immobilized on 96-well plates and LDL was added. Binding of LDL to decorin was analyzed using either an ApoB-detecting ELISA or a fluorometric cholesterol assay. Using ApoB-ELISA we found that B_{max} was positively correlated with LDL size ($r_s=0.78$, $p<0.01$), LDL cholesterol/ApoB ratio ($r_s=0.71$, $p<0.01$) and LDL cholesterol/triglyceride ratio ($r_s=0.85$, $p<0.01$). Corresponding negative relations were found with K_d. This means that bigger and more cholesterol-rich LDL bound better than small triglyceride-rich LDL. Normotriglyceridemic and hypertriglyceridemic individuals differed significantly in B_{max} (nTG = 0.69 vs. hTG = 0.54, $p<0.01$), K_d (nTG = 1.11 vs. hTG = 2.00, $p<0.01$) and LDL-size (nTG = 26.93 vs. hTG = 24.79, $p<0.01$) as well as in the composition of LDL. Similar results were obtained when LDL from 8 additional subjects were analyzed with the cholesterol assay. Similar results were also obtained when arterial-derived proteoglycans were used instead of decorin. In conclusion small, triglyceride-rich LDL particles bound less to decorin in this solid phase model than did big, cholesterol-rich LDL particles. These results suggest that the atherogenicity of small dense LDL may not depend on increased direct binding to extracellular matrix of the arterial intima, but may be related to other mechanisms, such as oxidative or enzymatic modification.

ACUTE INHIBITION OF CARNITINE PALMITOYL TRANSFERASE BY METHYL PALMOXIRATE (MP) IN VIVO DOES NOT INCREASE HEPATIC VLDL SECRETION.

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Introduction. VLDL secretion is generally believed to be substrate-driven, *i.e.* the supply of lipids for VLDL assembly determines the rate of VLDL secretion. Indeed, addition of fatty acids (FA) to hepatocytes leads to enhanced VLDL secretion. However, *in vivo* evidence for haptic FA content as being rate-limiting in VLDL secretion is lacking. We used methyl palmoxirate (MP), an inhibitor of carnitine palmitoyl transferase I, to acutely inhibit hepatic fatty acid oxidation and investigated whether the FA were subsequently rerouted into VLDL secretion. *Methods*. Male APOE*3Leiden transgenic were put on a Western diet and received an oral dose of 10 mg/kg MP in 0.05% methyl cellulose after an overnight fast. Plasma parameters were measured up to 8h after administration. VLDL secretion was measured using the Triton WR1339 method. Livers were collected and analyzed for lipid composition. *Results*. Administration of MP led to a 80-90% reduction in β-hydroxybutyrate compared to vehicle-treated mice (0.06 ± 0.03 vs 1.17 ± 0.52 mmol/L, respectively, $p<0.05$) upto 8 hours. Plasma FA levels were elevated in MP-treated mice compared to controls (1.37 ± 0.19 vs 1.04 ± 0.14 mmol/L, respectively, $p<0.05$).. Plasma glucose, cholesterol and TG levels were not affected. Surprisingly, no difference in VLDL-TG secretion was observed after Triton WR1339 administration (111.0 ± 13.3 vs. 121.7 ± 20.2 μmol TG/kg/h, respectively). Liver TG analysis revealed a 40% increase in liver TG content 8h after MP administration. *Conclusion*. Acute inhibition of fatty oxidation does not increase hepatic VLDL secretion.

THE INFLUENCE OF HEPATITIS C VIRUS INFECTION ON SERUM LIPID LEVELS

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The aim of this study was to investigate the influence of chronic hepatitis C virus (HCV) infection on the serum lipid levels of a variety of Japanese adult populations in the Kyushu area of Japan, some highly endemic for HCV infection. Between June 1999 and June 2001, serum total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, and alanine aminotransferase (ALT) level were measured in a total of 6,194 Japanese residents receiving annual public health examinations given by the local government for those aged 20 or older in K-Town (n = 2,410) and H-Village (n = 1,516), Fukuoka, in I-City (n = 1,166), Okinawa, and in G-Town (n = 1,102), Nagasaki, all in the Kyushu area, located in the southwestern part of Japan. Antibody to HCV (anti-HCV) was determined by enzyme-linked immunosorbent assay. Of the 6,194 residents surveyed, anti-HCV was detected in 454 (7.3 %): 33 (1.4 %) in K-Town, 331 (21.8 %) in H-Village, 123 (11.2 %) in G-Town and none in I-City. In K-Town, H-Village, and G-Town, anti-HCV positive residents were significantly older and had a significantly lower TC and higher ALT level than in those negative, in both sexes. In K-Town and H-Village, anti-HCV positive residents had a significantly lower LDL-C level than in those negative, in both sexes. In H-Village and G-Town, anti-HCV-positive male residents had a significantly lower HDL-C level than those negative. No statistically significant differences in serum lipid levels were found among the three study areas with anti-HCV positive residents, except I-City with no HCV-infected subject. From these findings that a lower cholesterol level was found in HCV-infected residents than in controls among the Japanese adult population, it is suggested that HCV infection is causal of lipid metabolism disturbances.

INHIBITION OF LIVER MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN MAY EXPLAIN HEPATIC STEATOSIS IN HCV-3 INFECTED PATIENTS

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Hepatic steatosis (HS) is a frequent histopathological finding of chronic hepatitis C, being more common in patients infected with HCV-3 genotype. The pathogenic mechanisms leading to HS in hepatitis C are still unknown and might be multifactorial. Recently HCV core protein was shown to inhibit the microsomal triglyceride transfer protein (MTP) which is crucial for hepatic VLDL assembly and secretion. Aim of this study was to correlate changes in hepatic MTP activity of patients with chronic HCV in relation to clinical and biological parameters. MTP activity was measured in needle liver biopsy by a sensitive fluorescent assay. The specific activity in each biopsy was expressed as % transfer/mg protein/hour. MTP specific activity ranged from 920 to 2293 (1472±315; n= 38) and did not exhibit any correlation with ALT or AST levels, histological activity and stage of liver disease. In HCV patients, a significant correlation was found between liver MTP activity and the HCV genotype: HCV3 patients had a significant lower MTP activity (1176±216) compared to patients with other HCV genotypes (1524±303; p= 0.02). HCV3 gruop had lower apoB (0,85±0,33 gr/l), total cholesterol (148±42,6 mg/dl) and LDL (66±30 mg/dl) levels compared to other HCV genotypes patients (apoB: 0,88±0,21 gr/l; cholesterol.: 170 ± 27mg/dl; LDL: 96±25 mg/dl) but these differences did not reach statistical significance. MTP activity was also measured in liver biopsies from 7 virus negative patients with non-alcoholic liver steatosis and in these cases MTP activity was significantly higher (1505±260; p=0,028) than in HCV3 cases, even though the two groups had similar degrees of HS. These results suggest that "viral-induced" HS in HCV-3 patients could be related to a genotype specific reduction in liver MTP activity.

APOLIPOPROTEIN E POLYMORPHISM IN NORTHWESTERN GREECE: FREQUENCY AND EFFECT ON LIPID PARAMETERS

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Apolipoprotein (apo) E gene polymorphism and its effect on serum lipid parameters were examined in a Greek population originating from northwestern Greece (N=555).
The allele frequencies were ε2: 6.3%, ε3: 80.7% and ε4: 13%. The ε4 allele frequency was higher in our population compared to that previously reported in individuals from other parts of Greece. ApoE polymorphism was associated with significant differences in serum lipid and lipoprotein levels. Particularly, individuals with the ε2 allele had higher serum triglyceride (TRG) and apoE levels and lower levels of total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C) and apoB compared to those with the alleles ε3 and ε4. However, the impact of the ε4 allele on lipid parameters seen in other populations was not observed in our population. Furthermore, the combination of both apoE polymorphism and serum apoE concentration explained a larger percentage of serum lipid variability than the polymorphism alone.
The results of our study suggest that ethnic differences, as well as alterations in serum apoE levels can significantly modify the relationship between apoE gene polymorphism and serum lipid variability.

DEVELOPMENT OF ATHEROSCLEROSIS IN APO-B100/CETP DOUBLE TRANSGENIC MOUSE

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The apoB/CETP mouse is a double transgenic mouse expressing human apolipoprotein-B100 and CETP resulting in a lipoprotein cholesterol distribution that resembles the human profile (Grass et al. J.Lipid Res. 1995;5.1082-91). Male mice (n=20) at the age of 25 weeks were put on a diet including cholesterol and sodium cholat. Atherosclerotic plaques were studied in formaldehyde fixed tissues by light microscopy in the whole mounted aorta after 11, 22 and 33 weeks on the fat diet. After 22 weeks plaques started to appear in the aortic arch in some of the animals. After 33 weeks on the diet plaques could be detected in the aortic arch as well as in the thoracic part of the aorta around the intercoastal artery branches in all mice. After 36 weeks on the fat diet the mice were given a normal chow diet during 5 weeks which did not seem to alter the aorta plaques (n=3). Plasma levels of cholesterol and triglycerides were also analysed. After the 33 weeks on the cholesterol enriched diet cholesterol and trigycerides levels were 13±2 mM and 0.5±0.1 mM respectively. After the 5 weeks on regular diet cholesterol levels decreased to 5.5±3.0 mM and triglycerides levels were 2±0.1 mM. In a previous study where the same mouse strain was given several types of western diet in the absence of sodium cholat no lesions were observed in the thoracic part of aorta and only small lesions in the sinus aorticus were found in some of the animals (Englund et al. 11th Int. Symp. on Atherosclerosis 1997 1.P.82). Thus, the apoB/CETP mouse fed a diet including both cholesterol and sodium cholat is a useful in-vivo model not only showing a human like lipoprotein profile but also ability to form atherosclerotic plaque. Furthermore this mouse model can be used for investigation of both progression and regression of atherosclerosis.

THE APOE2 KNOCK-IN MOUSE AS A MODEL FOR TYPE III HYPERLIPIDEMIA AND STEATOSIS

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Apolipoprotein E2 (APOE2) is a relatively common recessive allele, which is the main cause of Type III hyperlipidemia in man. The APOE2 knock-in mouse develops severe diet-induced hyperlipidemia. Similar to humans, it shows a marked cholesterol (Chol) and triglyceride (TG) lowering upon treatment with fibrate, an agonist of PPARα.
Using microarray analysis, we can use this unique mouse model to obtain a better understanding of the mechanisms underlying hyperlipidemia and to identify novel genes affecting lipid homeostasis.
APOE2 knock-in mice were either fed a high-fat diet or a high-fat diet in combination with fenofibrate. The mice were sacrificed at 2, 4, 7 and 21 days after start of the diet. As a control, we used chow fed APOE2 mice.
Blood parameters of these mice revealed a decrease in total Chol, and TG levels in response to fibrate. Analysis of the arrays showed that inflammatory genes were up regulated by the diet and down regulated by fibrate treatment. However, lipid metabolism genes were regulated similarly. Strikingly, regulation of these genes was seen already at two days after exposure to high fat diet.
Phenotypic analysis of these mice showed macrophage infiltration into the liver already 2 days after high fat feeding. In contrast, fat accumulation in the liver was noticed only after 4 days followed by a severe steatosis at one week. Fibrate treatment decreased the inflammation in the liver and abolished the steatosis. These observations correlate with the array data and indicate that fat accumulation in the liver may be preceded by macrophage infiltration.

Research supported by NHS, grant 2002B018

CORRELATIONS BETWEEN BRAIN HEMODYNAMICS AND LIPID METABOLISM INDICES IN ELDERLY ISCHEMIC STROKE PATIENTS

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Correlations between brain hemodynamics and lipid metabolism were studied in elderly post-ischemic stroke patients. 150 patients after ischemic stroke in carotid basin vessels (age of onset 50-64 years) and with cerebral atherosclerosis participated in the study between 2 months/one year after acute event. Methods: brain hemodynamics determined by duplex scanning of brain vessels on Sonoline Elegra (Siemens); lipid and lipoprotein contents determined on photometer 5010 Boehringer with assay kits from Sentenel CH (Italy). Elderly patients displayed dyslipoproteinemia (DLP) with increased contents of total cholesterol (6.09±0.17 mmol/l), low density lipoproteins (LDL) (3.01±0.05 mmol/l), apo B (1.41±0.05 mmol/l) and triglycerides (2.27±0.16 mmol/l) and decreased contents of high density lipoproteins (HDL) (0.95±0.04 mmol/l) and apo A (1.57±0.06). Profile of correlations between linear systolic blood flow velocity (LSBFV) in carotid and vertebro-basilar basin vessels and lipid metabolism indices in intact hemisphere was characterized by the presence of positive correlations between LSBFV in common carotid and anterior brain arteries and HDL contents (k=0.52 and k=0.48). In damaged hemisphere, negative correlations were found between LSBFV along vertebral artery and LDL content (k=-0.49). In intact hemisphere, the mean blood flow rate along common carotid artery correlated positively with HDL level (k=0.52) and negatively with cholesterol (k=-0.49) and LDL contents (k=-0.48) in median brain artery. In damaged hemisphere, the mean blood flow rate in common and internal carotid arteries correlated positively with HDL content (k=0.54 and k=0.60) and negatively with LDL content (k=-0.40 and k=-0.43) in vertebral and median brain arteries. Conclusion: In elderly post-ischemic stroke patients, brain hemodynamics indices in both intact and damaged hemispheres correlate positively with HDL level and negatively with cholesterol and LDL contents.

FAMILIAL HYPOBETALIPOPROTEINEMIA IS ASSOCIATED WITH FATTY LIVER DISEASE

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Introduction: Familial Hypobetalipoproteinemia (FHBL) is characterized by extreme low levels of apolipoprotein-B100 (apoB) containing lipoproteins due to the inability of truncated apoB's to be exported from the liver. Whereas the occurrence of fatty liver disease (FLD) has been put forward in small observational studies, the severity and consequences for chronic liver disease are unknown.
Methods: In order to establish relevance and severity of FLD in FHBL we performed a case control study in 41 subjects with clinical FHBL compared to 41 healthy, age and sex matched controls. Liver steatosis was quantified using ultrasonography. Additionally, history of risk factors for FLD was recorded and blood was collected for biochemical analysis.
Results: We found a significantly increased prevalence of liver steatosis in the FHBL group 54% (n=22) compared to 29% (n=12) in the control group (P=0.02). In addition, subjects in the FHBL group had more severe forms of steatosis (FHBL: 32% severe steatosis, 27% moderate and 41% mild steatosis; controls 0%, 33% and 67%, respectively). Liver biochemistry was comparable between groups. No differences were seen in factors known to be associated with steatosis such as BMI, glucose levels and use of alcohol.
Conclusion: FHBL is associated with an increased risk for FLD. Despite long-term existence of FLD in these subjects, no signs of steato-hepatitis were observed, indicating limited clinical relevance of FLD in FHBL subjects.

DYSLIPIDEMIA – THE MARKER OF PREECLAMPSIA IN THE PATIENT WITH HYPERTENSION IN PREGNANCY – OUR EXPERIENCE

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Arterial hypertension is a serious complication of 7-10% of pregnancies. Some clinical and experimental studies have brought interesting conclusions which showed the importance of the rational pharmacotherapy. By means of proper antihypertensive treatment it is possible to reduce the frequency of serious events such as preeclampsia and its complications. There is no medicament with ideal efficacy and safety which could be routinely recommended as antihypertensive drug of choice during pregnancy. During our cohort clinical study we had analysed the level of lipoprotein in pregnant women during the pregnancy and after delivery. Our team followed up 77 pregnant women during the period of 6 years. We formed a case study cohort. The patients were selected with the help of case analysis into special subgroups based on the classification of hypertension in pregnancy. The patients underwent internal and obstetric examination before medicament treatment was started. Magnesium was the drug of the first choice. With blood pressure higher than 140/90 mmHg acebutolol was added because of its pharmacokinetic and pharmacodynamic properties. The treatment was combined with other antihypertensives if blood pressure was not well controlled. We had observed in the subgroups of pregnant women with the development of preeclampsia the remaining level of lipoprotein (cholesterol, lipid). On the base of our cohort study we suppose the very important role of lipoprotein in the diagnosis, treatment and prognosis of preeclampsia and its complications.

THE DIFFERENCES BETWEEN LOW-DENSITY LIPOPROTEINS IN FAMILIAL HYPERCHOLESTEROLEMIA AND IN COMBINED HYPERLIPIDEMIA

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Small dense LDL are characteristic of familial hypercholesterolemia (FHCL) , while large LDL with lower density prevail in the blood of patients with familial combined hyperlipidemia (FCH). After ultracentrifugation, polar and nonpolar lipids were isolated from the LDL fraction by chromatography on a silicagel column and the content of inorganic phosphorus was measured. The content of double bonds (DB) was determined by ozone titration. In FHCL, LDL contained up to 70% DB of total serum lipids. The higher cholesterol (CL) content in LDL, the greater the content of DB. In FCH, the DB content was not greater than 50%. The higher CL content in LDL, the shorter lag-period and greater optical density of diene conjugates (DC) at 234 nm in reaction with Cu^{2+}. The greater the phospholipid (PL) content in LDL, the longer lag-period and less DC are formed. In all the patients, the higher CL content in LDL, the lower DB content in PL. The lower CL content and the greater the contents of TG, PL and DB in LDL, the longer lag-period and less DB are formed. It can be suggested that lag-period is the time during which active O_2 species oxidize fatty acids in PL and TG (primarily oleic acid) whose oxidation constant is considerably higher than that of arachidonic acid. After oxidation of the monoenic oleic acid, the remaining active O_2 species oxidize the polyenic arachidonic acid and form DC.

CHANGES IN SERUM LIPOPROTEINS SEPARATED BY HPLC AFTER LOADING OF DIACYLGLYCEROL OIL IN MAN.

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Diacylglycerol (DAG) oil, 70% of which is in the 1,3- isoform, has been shown to suppress postprandial elevations of serum TG compared with triacylglycerol (TAG) oil with a similar fatty acid composition and to promotes body fat reduction in man. We examined the difference in the kinetics of postprandial lipoproteins separated by gel filtration with HPLC between DAG and TAG after a single dose oral loading of these oils. Six male volunteers (average age 35.5y, BMI 24.9) ingested emulsified DAG or TAG oil with a similar fatty acid composition (30g/m^2 of body surface) in a double blind cross-over study. The effect of the DAG oil was particularly noticeable in the suppression of the level of chylomicron cholesterol in the RLP fraction (RLP-CM). Ordinary, small dense LDL-C separated by gel filtration is decreased after fat loading and this decrease in small dense LDL-C after DAG loading was significantly greater than that after TAG loading. These results suggested that DAG oil, compared with TAG oil, decreased lipoprotein formation in intestinal cells and consequently resulted in a decrease in the postprandial CM remnants. The mechanism of decreased small dense LDL by DAG loading is still unclear. However, a DAG substituted for a TAG-rich diet might lead postprandial lipid metabolism favorable for preventing coronary artery disease.

MEN WITH ISOLATED LOW FASTING HIGH DENSITY LIPOPROTEIN CHOLESTEROL HAVE A SLOWER REMOVAL OF TRIGLYCERIDES FROM CIRCULATION POSTPRANDIALLY COMPARED TO HEALTHY MIDDLE-AGED MEN

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Low levels of high density lipoprotein (HDL) cholesterol and disturbed postprandial lipemia are associated with coronary heart disease (CHD). We tested whether low levels of serum HDL cholesterol can influence the response to fatty meal.
Fifty two Greek men were divided into 2 groups: a) the low-HDL group [n=29, mean age 45(13) years] with HDL cholesterol < 40 mg/dl and a fasting triglyceride (TG) levels < 400 mg/dl; and b) the control group [n=23, mean age 51(9) years]. Plasma TG levels were measured before and 4, 6 and 8h after the fat load. A value of > 219 mg/dl was taken as an abnormal response to the fat load based on our previous studies.
The low-HDL group had higher TG levels at 4, 6 and 8h postprandially compared to controls (p<0.001). When both groups were matched for fasting plasma TG levels [100(31) vs. 95(27) mg/dl], they revealed higher TG levels at 8th h postprandially [154(78) vs. 105(38) mg/dl, (p=0.017)]. The former were characterized by lower levels of HDL cholesterol compared to controls [31(8) vs. 55(20) mg/dl, p=0.001)].
The low fasting HDL cholesterol levels seem to delay the rate of TG clearance postprandially, even in subjects with low fasting TG levels. Fasting plasma TG levels appear to be the primary determinant of the magnitude of postprandial lipemia.

POSTPRANDIAL FREE FATTY ACID METABOLISM IN NORMAL, DYSLIPIDEMIC AND TYPE 2 DIABETIC SUBJECTS: A RELATIONSHIP TO HEPATIC TRIGLYCERIDE METABOLISM?

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The delivery of free fatty acids (FFAs) to the liver is thought to be an important factor in the synthesis and secretion of very low density lipoprotein (VLDL) triglyceride, a major determinant of circulating triglyceride concentrations (TGs). However, previous studies have found no correlation between plasma FFAs and plasma TGs. Relatively little attention has been given to postprandial free fatty acid (FFA) metabolism and its potential contribution to hepatic TG production. We measured nocturnal and postprandial palmitate concentration and rate of appearance (Ra) in normotriglyceridemic control subjects (C, N=11); nondiabetic dyslipidemic subjects receiving n-3 fatty acids (3 g/d), niacin (3 g/d), the combination (3 g/d each) or placebo (DYS, N=28); and subjects with poorly controlled type 2 diabetes (DM, N=32). Nocturnal and postprandial palmitate Ra (area under the curve analysis) were higher in DYS and DM than in C (nocturnal 8.0±1.1 and 3.9±0.2 vs 1.8±0.4 $mmol \bullet m^{-2} \bullet h^{-1}$; postprandial 2.4±0.3 and 3.0±0.3 vs 1.1±0.1 $mmol \bullet m^{-2} \bullet h^{-1}$, all $P<0.001$). The relative postprandial abnormality in Ra was greater than the nocturnal abnormality in DM ($P<0.05$), but not in DYS. The postprandial palmitate concentration nadir was greater in DM and DYS than in C (35±3 and 23±3 vs 11±2 μmol/L, both $P<0.01$). There was no correlation between fasting FFA concentrations and fasting TGs in any group. However, using logarithmic regression, postprandial nadir palmitate concentrations correlated strongly with fasting TGs in C ($R=0.639$, $P<0.05$) and in DYS ($R=0.654$, $P<0.005$), but not in DM ($R=0.009$, P=NS) subjects. These results indicate that abnormalities in postprandial adipose tissue lipolysis make an inordinate contribution to around-the-clock FFA economy in insulin resistant states such as DM. They also suggest that postprandial FFA availability may be an important determinant of total hepatic FFA delivery for VLDL TG synthesis.

EFFECT OF POSTPRANDIAL LIPEMIA ON CORONARY ATHEROSCLEROSIS

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Purpose: To clarify the effect of postprandial lipemia to coronary artery disease(CAD), we investigated relationship between the concentrations of lipoproteins after the test meal load and the severity of the angiographic change in 35 consecutive male patients with definite or suspicion of CAD who underwent coronary angiography. Methods: The test meal consisted of normally consumed foodstuffs (599Cal; fat: protein: carbohydrate = 56.8%: 13.7%: 29.5%, cholesterol(Chol) 289 mg per 60 kg body weight, P/S ratio was 0.46) were fed. Serum lipids, lipoproteins and remnant like particles(RLP, by immunoabsorbent method) were measured in blood samples obtained in fasting state and at every 2 hr up to 6 hrs after the test meal load. Coronary scoring system(shown as CS, the method of Friesinger, et.al) was used for semiquantitative estimation of coronary atherosclerosis. Results: The concentrations of serum Chol, LDL-C and HDL-C did not change during 6 hr after the test meal load. On the other hand, those of serum TG, VLDL-TG and RLP-TG rose until 4 hr and then returned, but remained higher levels at 6 hr after the test meal load compared to those in fasting state. These changes were more prominent in patients with larger CS and so there were significant positive correlations between VLDL-TG and RLP-TG levels at 4 and 6 hrs after the test meal load, but between neither Chol levels nor LDL-C levels during 6 hr after the test meal load. Conclusion: VLDL-TG and RLP-TG at 4-6 hrs after meal had given some effects on development of coronary atherosclerosis.

DETERMINATION OF THE NUMBER OF DOUBLE BONDS IN POOLED UNSATURATED FATTY ACIDS AND OF COUPLED DOUBLE BONDS IN POLYENIC ACIDS FROM LOW DENSITY LIPOPROTEINS

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The content of double bonds (DB) in pooled fatty acids (FA) from LDL and the content of coupled DB in arachidonic acid (AA) were simultaneously measured in hyperlipidemic patients. DB in pooled FA and in AA were measured by automated ozone titration and spectrophotometric determination of diene conjugates in the presence of Cu^{2+}. The intake of O_3 upon oxidation of FA starts immediately, while diene conjugates form after a lag-period. The longer lag-period, the lower maximum optical density of DC, the less AA is oxidized by Cu^{2+}-induced active O_2 species. We believe that lag-period is the time during which active O_2 species oxidize DB of FA whose oxidation rate constants are higher than that of AA. The calculated oxidation constant for C18:1 oleic acid is $10^6 \cdot$ l/M.s and $2.4\ 10^5 \cdot$ l/M.s for C 20:4 AA. It can be suggested that the duration of lag-period reflects the content of oleic acid in triglycerides and phospholipids of LDL. At stable Cu^{2+} concentration , the higher the oleic acid content of LDL , the longer lag-period and the slower the formation of DC under the conditions of oxidant deficiency. Possessing the highest oxidation rate constant among all FA, oleic acid in the major biological acceptor of active O_2 species.

AUTOMATED KINETIC TITRATION WITH OZONE AND OXI-DATION CONSTANTS OF INDIVIDUAL FATTY ACIDS

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A device for automated titration with ozone has been designed. The kinetics of O_3 intake is determined by measuring O_3 concentration at the reactor output by UV-spectroscopy. The titrator allows one to determine the content of double bonds (DB) in fatty acids (FA) and express it in mM ozone used for oxidation. The method sensitivity is 10^{-9} M, the variation coefficient in a sample is 4.5%, and FA are titrated in chloroform. Stilbene solution with a DB content of 0.4-1.6 10^{-5} M is used as a standard.

The DB oxidation constant in FA varied in a wide range, being $1{,}1 \cdot 10^6$ l/M.s for ω-9 C 18:1 oleic acid, $6 \cdot 10^4$ l/M.s for ω-6 C 18:2 linoleic acid, and $2.4 \cdot 10^5$ l/M.s for the essential polyenic ω-6 C 20:4 arachidonic acid. The oxidation constant for the saturated C16:0 palmitic acid was only $6 \cdot 10^{-2}$ l/M.s. The kinetics of consecutively ozone-oxidized DB can be calculated for unsaturated linoleic and for the polyenic arachidonic FA. Similar to other active O_2 species, ozone oxidizes most actively the monoenic oleic FA. The degree of unsaturation and the concentration of monoenic FA can be determined in FA extracted by the method of Folch from serum lipids and low density lipoproteins. Unsaturation of FA in serum lipids changes considerably in patients with thermal burns.

STATINS DIFFERENTIALLY MODULATE THE Δ9, Δ6 AND Δ5 FATTY ACID DESATURASES IN MONOCYTIC AND HEPATOMA CELL LINES

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Statins, in addition to inhibit cholesterol synthesis, affect also the long chain polyunsaturated fatty acid (PUFA) production, e.g. they increase arachidonic acid levels in vitro and in vivo by enhancing linoleic acid metabolism, through enhanced Δ5 and to a smaller extent Δ6 desaturase activities.

Aims of our study were: 1) to evaluate the effect of statins on the Δ9 desaturase in cells incubated with [^{14}C] stearic acid, the substrate of this enzyme; 2) to compare the effects on the n-6 with those for the n-3 series using [^{14}C] α-linolenic acid (18:3 n-3) as substrate, in THP-1 and in HepG2 cells.

Results: 5μM simvastatin decreases Δ9 desaturase activity after incubation of THP-1 with [^{14}C] stearic acid with a significant difference in the product/precursor ratios 18:1/18:0 (1.119 vs 0.984, p=0.046) in control and treated cells.

As in the case of the n-6 series, THP-1 cells actively convert the substrate [^{14}C] α-linolenic acid (about 60%) to their products, and after treatment, Δ6 and Δ5 desaturases are also increased of 88% and 121% respectively. Simvastatin increases, in addition to the activity, also the Δ5 desaturase mRNA levels.

The enhancement of the conversion of 18C PUFA, i.e. α-linolenic acid, by simvastatin, is less pronounced in HepG2 vs THP-1 cells, whereas stearic acid conversion is increased in HepG2 cells, in contrast with the effect in THP-1. These findings reflect different features of lipid and fatty acid metabolism in these two types of cells. Lipids in THP-1 cells are mainly represented by structural phospholipids, i.e. they represent a saturable pool, whereas in HepG2 a considerable proportion of cell lipids are depot fats (triglycerides), i.e. a non saturable pool.

INFLUENCE OF PURIFIED N-3 FATTY ACID SUPPLEMENTATION ON THE LC-PUFA COMPOSITION AND METABOLISM.

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Background and aim: Nutrition plays an important role in the development of coronary heart disease (CHD). Regular consumption of n-3 polyunsaturated fatty acids of marine origin can lower blood pressure levels and reduce cardiovascular risk.
The purpose of this study was to investigate the influence of n-3 PUFA supplementation in diet on the fatty acid composition and metabolism.
Material and method: We studied 11 male, age 56,5±5 years. The BMI was 27,1±4 kg/m2, waist circumference 99,5±10 cm, SBP 102±14 and DBP 71±9 mmHg. All of them had coronary artery disease, were on statin therapy (simvastatin), nonsmoking and without diabetes. Drugs for secondary prevention (antiplatelet drugs, beta-blockers ACE-inhibitors) were recommended according to prevailing standard practice. In addition, patients were encouraged to adhere to recommended preventive measures, including a Mediterranean-style diet with high content of fruit, fish and fiber, and a relatively low content of saturated fat.
For 2 weeks the subjects were given a pharmaceutical preparation of highly purified and concentrated n-3 PUFA containing 90% n-3 PUFA per 1 g. Almost all the PUFA content is docosahexaenoic acid (DHA) and docoshexaenoic acid (EPA). The preparation was administrated at a dose of 1g/day. The blood samples were drawn and immediately frozen, and later fatty acids were determined by gas-liquid chromatography.
The PUFA metabolism was measured by metabolic index (ratio of metabolic products to baseline substrat and it reflects mainly the delta 6 desaturase activity) separately for n-3 and n-6 PUFA.
Results: The total amount of DHA and EPA increased almost half from r= 3,1±1,4 U to r=4,5±1,7 U ; p<0,05. None of the other measured fatty acids changed significantly.
The n-6 metabolic index decreased insignificantly from r= 0,46 to r=0,35, the n-3 metabolic index increased from r=11,4 to r=26,0 close but still statistically non significant (p=0,056).
Conclusions: The supplementation increased the level of docosahexaenoic acid (DHA) and docoshexaenoic acid (EPA) but did not influence in any significant way the calculated metabolic indexes of long chain polyunsaturated fatty acids.

LEPTIN INDUCES ABCA1 EXPRESSION OF MACROPHAGES AND REDUCES INTRACELLULAR CHOLESTERL ESTER CONTENT.

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Leptin is a circulating protein which is secreted mainly from adipose tissues. It is known that leptin regulates appetite and energy expenditure. And it is already reported that leptin deficiency increases hepatic triglyceride production. Metabolic syndrome, which includes obesity and dyslipidemia such as hypertriglyceridemia and hypo-HDL-cholesterolemia, is the main risk factor of coronary atherosclerosis. The purpose of this study is to elucidate the effect of leptin on ABCA1, which is a cell surface protein regulating lipoprotein metabolism, and on atherosclerosis. Methods: THP-1 cells were grown up with RPMI 1640 medium containing 10% fetal calf serum. THP-1 cells were differentiated into macrophages by incubation with same medium containing 100 nM tetradecanoyl phorbol acetate for 2 days. After differentiation into macrophages, cells were incubated with RPMI 1640 medium containing 0.5% fatty acid free bovine serum albumin and leptin was added to the medium to investigate the effect on mRNA expression. Results: THP-1 derived macrophages expressed more ABCA1 mRNA. When macrophages incubated with the medium containing leptin, ABCA1 mRNA increased significantly. When foam cells, which were prepared by incubation of macrophages with acetyl LDL, were incubated with the medium containing LXR agonist (TO-901317), ABCA1 mRNA increased and cholesterol ester (CE) efflux from foam cells increased, significantly. Conclusion: Leptin induced ABCA1 mRNA expression of macrophages, and this may prevent foam cell formation of macrophages by increasing CE efflux via ABCA1.

EFFECT OF ROSIGLITAZONE AND FISH-OIL DIET ON HEPATIC APO A-V AND A-IV mRNA IN ZUCKER FATTY RATS

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ApoA-V, a recently discovered apolipoprotein of hepatic origin, lowers plasma triglycerides. Little is known on the regulation of the apoA-V gene *in vivo*. ApoA-IV exerts a strong antiatherogenic effect. Plasma apoA-IV is elevated in diabetes and obesity. In obese hyper-triglyceridemic Zucker rats hepatic apoA-IV mRNA is increased.
We studied the effects of rosiglitazone, and of a hypolipidemic fish-oil diet on hepatic apoA-IV, A-V, and C-III mRNA in Zucker fatty rats. Adult male (fa/fa) Zucker fatty and (FA/-) Zucker lean rats (5-6/group) received a high-fat control diet (20% coconut oil), rosiglitazone (0.003% added to the diet for 3 weeks), a fish-oil diet (20% Menhaden oil), or fish-oil diet added to rosiglitazone. Rosiglitazone reduced plasma insulin, free fatty acids and triglycerides by about 50% in Zucker fatty rats.
Basal hepatic apoA-IVmRNA was increased to 153 ± 6% of lean control in fatty rats ($p < 0.01$), whereas apoA-V and apoC-III mRNA abundance were unaltered. Rosiglitazone increased apoA-V mRNA from 83 ± 13 to 143 ± 19 of lean control ($p<0.01$) and normalized apoA-IVmRNA in Zucker fatty rats. In lean rats rosiglitazone raised apoA-V mRNA to 174 ± 23 % of control ($p < 0.01$), whereas apoA-IV and C-III mRNA did not change. Fish-oil diet increased apoA-V mRNA from 83 ± 13 % to 158 ± 14 % ($p < 0.01$) in fatty rats. ApoA-IV mRNA decreased in both, Zucker fatty and lean rats on fish-oil diet.
Our data indicate that apoA-V does not contribute to the hypertriglyceridemia of Zucker fatty rats. Unlike apoA-IV, hepatic apo A-V expression was stimulated by rosiglitazone. Thus, apoA-V may participate in the triglyceride-lowering effect of this drug in Zucker rats.

PITAVASTATIN PROMOTES APOA- I PRODUCTION VIA SUPPRESSING PPARα PHOSPHOLYLATION IN HEPG2 CELLS

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Objective: There have been few reports describing the mechanism of HMG-CoA reductase inhibitors (statins) on HDL metabolism in vitro. We reported statins induced apoA I production in HepG2 cells at 13th ISA. In this paper, we report the mechanism of apoA- I production in HepG2 cells induced by statins.

Methods: HepG2 cells were treated with 0 to 30 μM pitavastatin, simvastatin or atorvastatin for 24hr containing 0.2% FCS, and mRNA of apoA- I and ABCA I were analyzed by RT-PCR. In order to examine the involvement of Rho protein to be geranylated by geranylgeranylpyrophosphate, HepG2 cells were also treated with Rho protein inhibitor C3T or RhoA kinase inhibitor Y27632 for 48hr containing 10% FCS. The apoA- I concentration in the medium was determined by ELISA. After treated with pitavastatin for 24hr, phospho-PPARα was determined with γ-P^{32}.

Results: Each statin induced mRNA expression of apoA- I and ABCA I dose-dependently: the order of the strength to induce the messages was pitavastatin>simvastatin>atorvastatin. ApoA I protein increased (170 or 140%) when HepG2 cells were treated with C3T or Y27632, respectively. Moreover, pitavastatin reduced PPARα phosphorylation.

Conclusion: It is said that an inhibition of Rho protein leads to reduce PPARα phosphorylation and increase apoA I mRNA. From above results, it is suggested that pitavastatin induces apoA I protein via inhibiting Rho protein and reducing PPARα phosphorylation.

IMPAIRED REVERSE CHOLESTEROL TRANSPORT IN PATIENTS WITH CORONARY CALCIFICATION

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Background. The definition of the presence of CAD in patients is usually defined by a history of CAD symptoms or events. The use of coronary calcification identifies asymptomatic individuals with calcified CAD. We investigated the phenotype/genotype interaction of 50 inflammatory genes, 50 lipid genes, and phenotypic markers in subjects with and without coronary calcification.

Methods. 300 subjects without coronary calcification (EBT-) and 300 with coronary calcification (EBT+) are being recruited. To date 231 subjects have completed data collection. Fasting blood was analyzed for triglycerides (TG), total cholesterol (TC), LDL-C and HDL-C by enzymatic methods, 7 LDL and 5 HDL subclasses (%) by S3-Grandient Gel Electrophoresis, Lp(a), hs-CRP, and insulin by immunoassay.

Results. (mean ±SD)	**EBT+**	**EBT-**	**p**
N	144	87	
Waist to Hip	0.90±0.05	0.91±0.06	0.17
LDL-C mmol/L	3.31±0.80	3.21±0.83	0.35
HDL-C mmol/L	1.15±0.29	1.17±0.32	0.59
TG mmol/L	1.50±0.84	1.50±0.76	0.98
LDL IIIa+b (%)	25.5±11.4	23.7±11.2	0.24
HDL2b (%)	14.3±5.6	16.1±7.2	0.03
Hcy	9.1±2.8	9.1±2.1	0.99

Conclusion. Lipid and homocysteine values were not different between groups. Despite similar HDL-C values, subjects with coronary calcification evidenced significantly lower distribution in the HDL2b region. Disorders of reverse cholesterol transport may predispose to coronary calcification despite "normal" HDLC values.

CHANGES IN THE HDL-CHOLESTEROL LEVELS DURING AND AFTER ACUTE MYOCARDIAL INFARCTION

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Low HDL cholesterol level in the blood, increase the risk of unwanted coronary events at the patients with a verified CAD.Numerous studies have shown that the level of the serum lipids, measured in the first 24 hours of the acute myocardial infarction (AMI), in fact is the basal lipid level, which is liable to changes immediately after AMI, and get back to its basal value within the next 6-12 weeks.

In order to confirm if there are changes in the lipid profile,at 230 middle aged (59.87 ± 13 years old) , mostly males (66.5%) patients with AMI, a follow up of the HDL cholesterol level was performed, in vein blood and determined by standard enzymatic methods in different time intervals after AMI (24 hours, 3-7 days, 10-14 days,30-60 days, 60-90-days).

Results : The patients with AMI have had a lower initial HDL cholesterol level, which showed a tendency to decrease three days after AMI, and to be gradually "normalized" after 60-90 days, i.e. not only turning back the HDL cholesterol values to the initial level, but their overcoming too.

The average value of HDL cholesterol, checked after 60-90 days after AMI, is overcoming that basal value in a positive sense, but it was further on higher than the wanted aim of 40mg/dl (1.03mmol/l).

Concludion : The optimal time for determining HDL cholesterol at the patients with AMI, are the first 24 hours, since in that period there is a relevant decrease of the HDL cholesterol level in the blood. The values of the lipid profile acquired at that period, should be considered as basal.

LERCANIDIPINE REDUCES FREE CHOLESTEROL-INDUCED CYTOTOXICITY

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Excess intracellular free cholesterol (FC) is cytotoxic to many cells types and it has been hypothesized that accumulation of FC is a mediator of macrophage death *in vivo* and contributes to the atherosclerotic lesion progression. Excess cellular FC generated through the hydrolysis of cytoplasmatic cholesteryl ester (EC) is esterified by ACAT enzyme or may be transported to the plasma membrane where it induces a variety of responses, including increased phospholipid synthesis, cell death and FC crystal formation. Lercanidipine is a new calcium antagonist with high lipophilicity. We previously observed that lercanidipine reduced both cholesterol esterification and free cholesterol accumulation in macrophages. These effects do not involve a Ca^{++} antagonist action, but are related to the lipophilic characteristic of the molecule and these observations indicate that the drug may interfere with cellular cholesterol trafficking. The aim of this study was to examine lercanidipine activity in preventing FC-induced cytotoxicity in J774 macrophage foam cells. J774 were cholesterol-enriched using 100μg/ml acetylated low-density lipoprotein (AcLDL) and 250μg/ml FC/phospholipid dispersions (FC/PC). After 48h of incubation cells monolayer were labeled with 1μCi [^{3}H]-adenine. To evaluate the effect of lercanidipine on FC-induced cytotoxicity J774 macrophage foam cells were induced to accumulate excess intracellular FC by incubation with an ACAT inhibitor. Cellular cytotoxicity was measured by cellular release of [^{3}H]adenine. The ACAT inhibitor induced a 3-fold increase of adenine release. No effect was observed with lercanidipine. Moreover, ACAT-inhibited J774 foam cells incubated in the presence of lercanidipine 10μM showed a reduction in adenine release from cells by almost 30%. We conclude that lercanidipine may play a role in protecting cells from toxic concentrations of free cholesterol.

PROBUCOL INHIBITION OF ATP-BINDING CASSETTE A1-MEDIATED CELLULAR LIPID EFFLUX

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ABCA1 mediates lipid efflux from cells to lipid-poor apolipoproteins. In this study we characterized the effect of the lipid lowering drug probucol on ABCA1-mediated lipid efflux in culture cells. We incubated ABCA1 expressing J774 macrophages with probucol and then promoted cholesterol efflux to apoA-I for 4h. Probucol inhibited in a time and concentration dependent manner cellular efflux up to 80%. Similar results were observed on peritoneal mouse macrophages and human fibroblasts. In all cells the release of phospholipid, a process specifically mediated by ABCA1, exhibited a similar degree of inhibition. Concomitant with the reduction of lipid efflux, probucol inhibited the ABCA1-mediated cholesterol enrichment of plasma membrane domains. In Fu5AH hepatoma cells which contain high levels of SR-BI we demonstrated no effect of probucol on SR-BI-mediated efflux to HDL. Fluorescent confocal microscopy demonstrated that probucol decreased the translocation of ABCA1 from intracellular pools to the plasma membrane in J774. Consistent with the inhibitory effect on ABCA1 translocation to the plasma membrane, probucol reduced cell surface specific ^{125}I-apo-AI binding. We conclude that probucol shows a selective inhibition of ABCA1-mediated cholesterol efflux without influencing SR-BI-mediated efflux. This effect involves an inhibitory effect of the drug on ABCA1 translocation to the plasma membrane and may explain the ipoalphalipoproteinemic effect of probucol in human.

ENHANCED CHOLESTEROL EFFLUX FROM J774 MACROPHAGES AND FU5AH HEPATOMA CELLS TO SERUM FROM CETP DEFICIENCY

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Backgrounds : It has been demonstrated that large HDL particles from CETP deficiency is less effective in promoting cholesterol efflux from lipid-loaded macrophages, however, little speculation has taken place concerning the detailed pathways of cellular cholesterol efflux. Methods : Sera from 3 homozygotes, 3 heterozygotes CETP deficiency (intron 14 splicing defect (In14)) or normolipidemic control individuals were compared using two different cell systems. Results : In the In14 homozygotes, CETP activity was undetectable and presented with marked hyperalphalipoproteinemia (mean HDL-C 150mg/dl). On the other hand, in the In14 heterozygotes (mean HDL-C 103mg/dl), CETP mass was decreased to 66 % of controls. In the SR-BI-rich Fu5AH cell system, sera from homozygotes CETP deficiency showed higher cholesterol efflux than did control sera ($p<0.05$). In the J774 macrophage cell system, pretreatment with liver X receptor (LXR) alpha agonist (TO-901317; 5 uM), which upregulates ABCA1, induced an increase in the efflux to In14 heterozygotes sera as well as to control sera by 183%, whereas it had little effect on cholesterol efflux to In14 homozygotes sera. Conclusions : We showed for the first time that cellular cholesterol efflux activity both in ABCA1-expressing J774 cell and SR-BI rich Fu5AH cell system are differentially regulated by altered HDL subpopulations found in CETP deficiency. Cholesterol acceptor function in these cell systems appeared to be preserved in ABCA1 dependent pathway in heterozygotes, but SR-BI dependent pathway in homozygotes.

MULTIPLE GENE OVEREXPRESSION LEADS TO A DRAMATIC INCREASE OF CHOLESTEROL EFFLUX FROM MACROPHAGES

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Accumulation of cholesterol in macrophages is a key feature of atherosclerosis. The removal of excess cholesterol by cholesterol efflux is considered a promising approach for the prevention and treatment of atherosclerosis. We tested the simultaneous overexpression of several genes on [^{3}H]cholesterol efflux from RAW 264.7 mouse macrophages. To achieve high efficiency transfection of multiple genes, a new method for transient transfection of macrophages was developed. This method was based on our finding that poor efficiency of transfection of macrophages results from methylation silencing of the CMV promoter rather than from a low efficiency of DNA transfer. Consequently, transfection with DEAE-Dextran followed by demethylation using the epigenetic modifier, 5-azacytidine, resulted in >90% efficiency of transfection. The effect on cholesterol efflux following transfection or activation of the following genes was tested: ABCA1, caveolin-1, CYP27A1, SR-B1, apolipoprotein A-I (apoA-I) and PLTP. When tested individually, ABCA1 (activated by treatment with 1 μM T0901317) and SR-B1 were the most active and resulted in approximately 3-fold increase in cholesterol efflux to 20 μg/ml human apoA-I or 3 % human plasma. Among combinations of two genes, a combination of ABCA1 and SRB1 was the most active resulting in a 4-fold increase in cholesterol efflux. Among three gene combinations a combination of ABCA1, SR-B1 and CYP27A1 resulted in a 10-fold increase of cholesterol efflux to apoA-I. The most active combination was simultaneous overexpression of ABCA1, SR-B1, CYP27A1 and PLTP, which resulted in a 56-fold stimulation of the efflux to apoA-I compared with mock-transfected cells. We conclude that simultaneous overexpression of multiple genes may overcome several successive rate-limiting steps and lead to a dramatic stimulation of cholesterol efflux.

STEROL 27-HYDROXYLASE AS PART OF THE LIVER X RECEPTOR-REGULATED SYSTEM PROMOTING STEROL EFFLUX FROM MACROPHAGES

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Efflux of cholesterol from macrophages is accomplished by means of several proteins: the ATP-binding cassette transporters, namely ABCA1, the bi-directional scavenger receptor SR-BI and the sterol acceptor apoE. Interestingly all these genes are positively regulated by agonists of liver X receptor (LXR). Recent evidences support the role of sterol 27-hydroxylase (CYP27) as a potential contributor to sterol efflux from macrophages. By gene expression profiling in human monocyte-macrophages derived from peripheral blood we found that CYP27, apoE, SR-BI and LXRα are upregulated during the differentiation time. Therefore the aim of our studies was to investigate whether CYP27 is a novel LXR regulated gene. We found that CYP27 mRNA level is increased by the LXR agonist T0901317 in blood-derived monocyte-macrophages but not in PMA-differentiated THP-1, probably because the latter cell line expresses insufficient amounts of LXR. In transient transfection assays we found that the human CYP27 promoter is transactivated by LXR agonists only when a LXR expression plasmid is co-transfected and to a lesser extent in comparison to a reporter system carrying a *bona fide* LXR responsive element. The computational analysis of the CYP27 promoter failed to reveal the presence of a putative LXR responsive element therefore it can be hypothesized that LXR affects CYP27 transcription through an indirect mechanism that needs to be further characterized. Our findings highlight the potential role of CYP27 as part of the cellular response triggered by LXR activation aimed at promoting sterol efflux from macrophages.
A. Vigil is a fellow supported by a Marie Curie training program (EC)

3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE INHIBITORS ENHANCE HDL-MEDIATED CHOLESTEROL EFFLUX FROM MACROPHAGES IN PART THROUGH ABCA1 PATHYWAY

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The mechanisms of increased HDL cholesterol induced by statin therapy were not well understood. The present study was performed to examine effect of statins on macrophage expression of molecules involved in HDL-induced cholesterol efflux. RAW macrophages or peritoneal resident macrophages from PPARα null mice were incubated for 24 hours with or without pitavastatin or atorvastatin. Protein expression of SR-B1 and caveolin-1 and mRNA expression of ABCA1, PPARα and LXRα were investigated. RAW cells treated with statins were further incubated for 24 hours with acetylated LDL in the absence or presence of HDL_3 to test statin-induced cholesterol efflux. Statins increased the expression of SR-B1 and caveolin-1 in a dose- dependent manner in response to PPARα expression. ABCA1 expression was increased by low concentrations of statins, but decreased by its high concentrations. This statin effect on ABCA1 was also found in PPARα null macrophages and acted in concert with LXRα expression. Statins significantly enhanced HDL- induced inhibition of cellular cholesterol accumulation. These results suggest that statins increase macrophage expression of molecules involved in HDL cholesterol efflux. Above all, low concentrations of statins increase ABCA1 expression and cholesterol efflux from macrophages possibly in relation to LXRα.

EFFECT OF ROLIPRAM ON ATP BINDING CASSETTE TRANSPORTER A 1 AND CHESTEROL EFFLUX IN THP-1 MACROPHAGE-DERIVED FOAM CELL

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To study the effect of rolipram on ATP binding cassette transporter 1 and cholesterol efflux in THP-1 macrophage-derived foam cell. After exposure of the cultured THP-1 macrophage-derived foam cell to rolipram for different time, cholesterol efflux and ABCA1 mRNA and protein level were determined by FJ-2107P type liquid scintillator and reverse trancriptase-polymerase chaim reaction and western blot, respectively. Rolipram promotes cholesterol efflux in THP-1 macrophage-derived foam cell with time; RT-PCR and western blot showed that exposure of the cultured THP-1 macrophage-derived foam cell to rolipram for different time, resulted in increasing in the expression of ABCA1 mRNA and protein in THP-1 macrophage-derived foam cell with time, respectively; High performance liquid chromatography and enzyme immunoassay showed that exposure of the cultured THP-1 macrophage-derived foam cell to rolipram for different time, resulted in decreasing Cholesterol and cholesterol ester in THP-1 macrophage-derived foam cell with time and increasing cAMP level in THP-1 macrophage-derived foam cell with time. Rolipram promotes cholesterol efflux and increases the expression of ABCA1 in THP-1 macrophage-derived foam cell. cAMP phosphodiesterase 4 inhibitors may provide a novel strategy for treatment of atherosclerosis.

MODERATE ALCOHOL CONSUMPTION INCREASES CHOLESTEROL EFFLUX MEDIATED BY ABCA1

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Moderate alcohol consumption increases HDL cholesterol, which is involved in reverse cholesterol transport. The aim of this study was to investigate the effect of moderate alcohol consumption on cholesterol efflux, using J774 mouse macrophages and Fu5AH cells, and on other parameters in the reverse cholesterol transport pathway.
Twenty-three healthy men (45-65 years) participated in a randomized, partially diet-controlled, cross-over trial. They consumed four glasses of either whisky (40 g alcohol) or water during 17 days. After 17 days of whisky consumption the capacity of serum to induce ABCA1-dependent cholesterol efflux from J774 mouse macrophages was increased by 17.5% ($p= 0.027$) as compared to water consumption and the capacity of plasma to induce cholesterol efflux from Fu5AH cells increased by 4.6% ($p=0.002$). Pre β-HDL, apoA-I and lipoprotein AI:AII increased by respectively 31.6, 6.2 and 5.7% ($p<0.05$) after whisky consumption as compared to water consumption. Changes of cholesterol efflux from cAMP-stimulated macrophages correlated significantly ($r=0.65$; $p<0.05$) with changes of apoA-I, but not with changes of pre-β HDL ($r=0.30$; $p=0.18$). Cholesterol efflux capacity of serum from lean men was 7.7% higher than from overweight men ($p=0.02$). In conclusion, this study shows that moderate alcohol consumption increases the capacity of serum to induce cholesterol efflux from J774 mouse macrophages, which may be mediated by ABCA1.

REGIONAL VARIATIONS IN ABC TRANSPORTER EXPRESSION ALONG THE MOUSE INTESTINAL TRACT

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The ATP-binding cassette family of proteins comprise a group of membrane transporters involved in the transport of a wide variety of compounds, such as xenobiotics, vitamins, lipids, amino acids and carbohydrates. Determining both their regional expression patterns along the intestinal tract and factors modulating this expression pattern will further characterize their transport functions in the gut. The mRNA expression levels of murine ABC transporters in the duodenum, jejunum, ileum, and colon were examined using the Affymetrix Mu74v2 GeneChip set. Eight ABC transporters (Abcb2, Abcb3, Abcb9, Abcc3, Abcc6, Abcd1, Abcg5, and Abcg8) displayed significant differential gene expression along the intestinal tract, as determined by two statistical models (a global error assessment model and a classical ANOVA, both with a $p<0.01$). Concordance with semi-quantitative real-time PCR was high. The cellular location of Abcc3 (MRP3) was examined by immunohistochemistry. Staining revealed that the protein is consistently expressed in the basolateral compartment of enterocytes along the anterior-posterior axis of the intestine. Furthermore, the intensity of the staining pattern concords with the expression profile. This agrees with previous findings in which the mRNA, protein and transport function of Abcc3 were increased in the rat distal intestine. These data reveal regional differences in gene expression profiles along the intestinal tract and demonstrate that a complete understanding of intestinal ABC transporter function can only be achieved by examining the physiologically distinct regions of the gut.

TWO PREVALENT MUTATIONS IN THE ATP-BINDING CASSETTE TRANSPORTERS *ABCC6* IN JAPANESE PATIENTS WITH PSEUDOXANTHOMA ELASTICUM (PXE)

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Pseudoxanthoma elasticum is a heritable disorder of the connective tissue characterized by progressive calcification of elastic fibers in skin, retina, and the cardiovascular system. Recently, the ABCC6 gene is identified as being responsible for PXE. A defective type of ABCC6 gene was determined in 16 Japanese patients with PXE from two separate districts of Hokuriku (central Japan, Sea of Japan side) and Tokyo. We examined each of 31 exons and flanking intron sequences by PCR methods (SSCP screening and direct sequencing). Four different mutations in the ABCC6 gene were identified in 28 alleles out of total 32 alleles (4 alleles have not been identified yet). The most common mutation was one base deletion (2542delG) in exon 19 (17/32 alleles; 53%). C to T substitution in exon 9 (Q378X) was found in 8/32 alleles (25%). We also found two novel missense variants in exon 26 (R1221C; 1/32 allele) and exon 29 (R1357W; 2/32 alleles). We have found neither R1141X nor ABCC6del23-29 mutations which have been reported to be highly frequent in Caucasian population. No significant correlation could be established between the clinical manifestations and disease-causing mutations in the patients with PXE. Further investigations for a nation-wide survey of Japanese patients with PXE are needed to confirm our results.

THE EFFECT OF STATINS ON INTESTINAL ATP BINDING CASSETTE PROTEINS G5 AND G8

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The ATP binding cassette proteins (ABC) G5 and G8 have been shown to regulate cholesterol absorption in the intestine and excretion from the liver. The intestine is an important organ in cholesterol synthesis but there is little information on the effect of statin therapy on the regulation of intestinal cholesterol. The present study was undertaken to examine ABC G5 and G8 levels in hypercholesterolaemic patients who were being treated with statins and to compare these with levels in normocholesterolaemic subjects. Seventeen subjects on statin therapy and thirty non-statin treated control subjects who were undergoing routine gastroscopy and were found to have histologically normal duodenal biopsies were compared. Ethics committee approval was obtained and all subjects gave informed consent. ABC G5 and G8 mRNA levels were measured using RT-PCR and expressed in relation to the GAPDH housekeeping gene. Serum lipids were determined by routine laboratory methods. Subjects in the two groups were of similar age and BMI. Serum cholesterol was not significantly different between the groups being 4.7±0.9 for the statin group and 4.3±1.1 for the controls (mean±sd). Serum triglycerides were not significantly different (median 1.9 controls and 3.7 for statin-treated patients). Intestinal ABCG5 (0.20±0.09 and 0.16±0.09) and ABCG8 (0.077±0.03 and 0.088±0.05) were similar in the two groups. There was a strong negative correlation between ABCG5 and serum cholesterol ($r=-0.7$, $p<0.001$) and ABCG8 and serum cholesterol ($r=-0.78$, $p<0.0001$) in the control group but no correlation with either ABCG5 or G8 in statin treated patients. The strong negative correlation between ABCG5 and G8 and serum cholesterol shows that these proteins play a major part in regulating serum cholesterol. Statin therapy appears to reduce cholesterol independently of the ABCG5 and G8 pathways.

PARAOXONASE 55/192 ALLELE FREQUENCY DISTRIBUTION IN THE HUNGARIAN GENERAL POPULATION AND HYPERCHOLESTEROLAEMIC GROUP

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Paraoxonase (PON1) enzyme is one of the components of HDL responsible for the prevention of lipid peroxides' accumulation in LDL. Numerous epidemiological studies have been carried out on risk groups so far but only a few data are available on genetic polymorphism predisposing cardiovascular diseases in the general population. The molecular basis of PON1 activity is a single nucleotide change resulting in Gln (Q)→Arg (R) amino acid substitution at position 192. The PON1 192R isoform is found to be less effective at hydolising lipid peroxides than the Q isoform thus PON1-192R allele is positively associated with coronary heart diseases. The Met (M) → Leu (L) interchange at position 55 of the PON1 gene is thought to modulate the effect of the 192 polymorphism independently. PON1 55/192 polymorphism studies were carried out on DNA samples from 1185 individuals (551 males, 629 females, 5 unknown) representing the Hungarian general population age and sex distribution, and 117 patients (57 males, 60 females) with kidney failure admitted to the health care program of the University using LightCycler real time PCR technology. The frequency of PON55 and PON192 genotypes among patients were found as PON55LL: 43.59%; PON55LM: 46.15%; PON55MM: 10.26%; PON192QQ: 52.99%; PON192QR: 39.32%; PON192RR: 7.69%., while the distribution of PON55 and PON192 alleles were PON55L: 66.67%; PON55M: 33.33%; PON192Q: 72.65%; PON192R: 27.35%. In the reference group the PON 55/192 genotype and allele frequencies were the following PON55LL: 41.05%; PON55LM: 46.88%; PON55MM: 12.08%; PON192QQ: 50.68%; PON192QR: 40.71%; PON192RR: 8.61%; PON55L: 35.52%, PON55M: 64.48%, PON192Q: 71.03%; PON192-R: 28.97%.

SERUM PARAOXONASE ACTIVITY IN HYPERTENSIVE MENOPAUSAL WOMEN

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Paraoxonase (PON) is an enzyme associated with HDL in human serum that inhibits LDL oxidation, which is an important step in atherogenesis. PON levels are lower in patients with myocardial infarction and with diabetes mellitus, suggesting an important role for serum PON in cardiovascular diseases. The purpose of the study was to examine the PON activity, lipids levels, body mass and blood pressure (BP) values and to calculate some atherogenic indexies in menopausal women. One group (A) of women was examined in The Cardiological Preventive Study for 40 Years Aged Men and 50 Years Aged Women, and the younger women (group B, aged 45 yrs) became from general population. Among 106 women of group A 72 % had hypertension complicated by dyslipemia, obesity and CHD or DM. The rest of A and 70% of group B (n=90) were healthy. Nearly half of them have smoked ~20 cigarettes daily. The hormone replacement therapy (HRT) used 34% of group A. PON activity was determined using paraoxon as the substrate. PON activity differed between two groups (785±103 vs. 274±46 U/ml). Lipids levels, systolic BP and atherogenic indexies values were higher in hypertensive women. Smokers had lower PON activity than non-smokers, and HRT increased PON activity and HDL levels in both of groups. A negative correlation between PON and diastolic BP was observed in menopausal women. The associations PON activity and total cholesterol and apoA1 levels existed in younger women. We suggest that PON overproduction is possible in treated hypertensive menopausal women with obesity and dyslipemia. Probably ACE inhibitors during the treatment increased PON expression and limited oxidative stress and vasculars wall injury in these women.

HDL FROM PATIENTS WITH CHRONIC HEART FAILURE INCREASES APOPTOSIS IN HYPOXIC RAT CARDIAC MYOCYTES

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Background: In the addition to reverse cholesterol transport, HDL prevents the endothelial cell death induced by Oxy-LDL or tumour necrosis factor alpha (TNFalpha). However, little is known about the anti-apoptotic effect of HDL in cardiac myocytes subjected to hypoxic injury.
Objective: The study was carried out to examine the effect of HDL from patients with chronic heart failure (CHF) on the process of apoptosis in cardiac myocytes.
Methods: HDL fraction (1.063 - 1.210 g/mL) was isolated by ultracentrifugation from sera of 5 healthy subjects and 5 patients with stable, severe CHF (age: 60 +/- 3 years, LVEF: 27 +/- 2 %, NYHA class III-IV). CHF patients demonstrated elevated serum levels of high sensitivity C-reactive protein (7.2 +/- 2.1 mg/dL), TNFalpha (5.4 +/- 0.7 pg/mL) and homocysteine (17.2 +/- 1.3 mmol/L). Primary cultures of neonatal rat cardiac myocytes were subjected to hypoxia (5% CO2 and 95% N2) during 7 hours in the presence or the absence of HDL (1 mg/mL). The activity of caspase-3 as a marker of apoptosis in rat cardiac myocytes was determined using spectrophotometry (AC-DEVD-AMC assay kit, Sigma RB1).
Results: HDL from CHF patients were characterized by significantly lower levels of cholesterol ($p<0.01$), phosphatydylocholine ($p<0.02$) and sphingomyeline ($p<0.005$) when compared to HDL fraction isolated from healthy subjects. The activation of caspase-3 in cardiac myocytes exposed to hypoxia in the presence of HDL from CHF patients was increased by 4 times (range: 3.5 - 6 times) when compared to the caspase-3 activation in hypoxic cardiac myocyte cultured with HDL from healthy subjects ($p<0.05$).
Conclusion: In our study we have demonstrated for the first time that HDL from CHF patients posses the pro-apoptotic properties.

TRANS FATTY ACIDS INDUCE APOPTOSIS IN HUMAN ENDOTHELIAL CELLS

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An increasing body of evidence from human specimens suggests that apoptosis is a major factor involved in the physiopathology of atherosclerosis. It has been proposed that apoptotic cell death contributes to plaque instability, rupture and thrombus formation. Epidemiological and clinical studies suggest that the intake of trans fatty acids increases the risk of coronary heart disease. The present study was designed to investigate the hypothesis that trans fatty acids can induce apoptosis of human umbilical vein endothelial cells (HUVEC). To test this hypothesis, apoptosis in HUVEC was measured with the use of 0.1, 1.0 or 5.0 mM solution of trans elaidic acid (t-18:1) or linoelaidic acid (t,t-18:2) for 24 hours. Apoptosis was detected with the use of the TdT-mediated dUTP nick end labelling assay (TUNEL) and of cellular binding of anexin V and propidium iodide uptake measurement. Moreover, active Caspase-3, cleaved PARP (polymerase poly-ADP-rybose) and Bcl-2 levels were also measured in the cell lysate. Both acids studied induced apoptosis in HUVEC in the dose-dependent manner through their effect on Caspase-3 activity. It was proven that pretreatment of HUVEC with trans fatty acids was connected with an increased level of active Caspase-3 and of cleaved PARP, which are formed under the action of this enzyme. No effect of the acids studied on the level bcl-2, an apoptosis inhibiting protein, was found. It is worth emphasising that linoelaidic acid proved to be a stronger apoptosis inducer in endothelial cells. In the case of elaidic acid (5mM), 21% anexin-positive cells, 40% cells coloured by propidium iodide, and 40% TUNEL-positive cells were found. On the other hand, in the case of linoelaidic acid (5mM) 30% anexin-positive cells 52% propidium iodide-coloured cells, and 65% TUNEL-positive cells were found. The present study suggests that changes in the cellular lipid environment may play a role in the damaging and death of vascular endothelial cells in atherosclerosis.

IMPAIRED RECYCLING OF APOLIPOPROTEIN E-4 IS ASSOCIATED WITH INTRACELLULAR CHOLESTEROL ACCUMULATION

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After internalization of triglyceride-rich lipoproteins (TRL) in hepatoma cells, TRL particles are immediately disintegrated in the early endosomal compartment. This involves the targeting of lipids and apoprotein B along the degradative pathway and the re-secretion of TRL-derived apoE through recycling endosomes. This is followed by the concomitant association of apoE and cellular cholesterol with high-density lipoproteins (HDL). Since epidemiological data show that apoE3 and apoE4 have differential effects on HDL metabolism, we investigated whether the intracellular processing of TRL-derived apoE4 is altered in comparison to apoE3.
In this study we demonstrate by radioactive and immunofluorescence uptake experiments that internalization of TRL-derived apoE4 is increased compared to apoE3 in hepatoma cells. Pulse chase experiments revealed that HDL-induced recycling but not disintegration of TRL-enriched with apoE4 is impaired compared to apoE3. Most importantly, the decrease in HDL-induced apoE4 recycling is associated with a reduced cholesterol efflux. Similar studies performed in Tangier fibroblasts show that apoE recycling does not depend on ATP binding cassette transporter A1 activity. These studies provide evidence that impaired recycling of apoE4 could interfere with intracellular cholesterol transport and contribute to the pathophysiological lipoprotein profile observed in apoE4 homozygotes.

ENDOTHELIAL LIPASE (EL) AND SCAVENGER RECEPTOR CLASS B, TYPE I (SR-BI) CONTRIBUTE TO PHOSPHOLIPID AND FATTY ACID SUPPLY IN THE CEREBROVASCULATURE

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Polyunsaturated fatty acids (PUFA) are necessary for normal development and function of the human brain. Cerebral PUFAs can originate from the free FA plasma pool or lipoprotein-associated lipids. Therefore we investigated pathways that facilitate uptake of lipoprotein-associated phospholipids (PL), subsequent hydrolysis and import of liberated PUFAs by primary porcine brain capillary endothelial cells (pBCEC), an *in vitro* model of the blood-brain barrier (BBB). Endothelial lipase (EL) has been found to be a key enzyme in high-density lipoprotein (HDL) metabolism in mice, catalyzing hydrolysis of HDL-associated PL. During the present study we demonstrate EL expression by pBCECs on mRNA and protein level. Immunohistochemical characterization of EL expression revealed predominant staining at or near brain capillaries. Treatment of pBCECs with PPAR- and LXR-agonists significantly regulated the amount of EL mRNA levels. PL-tracer experiments revealed hydrolysis of the tracer and liberation of [^{14}C]-20:4 in a time dependent manner. One potential pathway that could fuel HDL-derived PL to the cerebral microvasculature is the selective uptake pathway. In line, we could identify the expression of scavenger receptor BI (SR-BI) by pBCECs, one of the receptors mediating selective lipid uptake. Overexpression of SR-BI resulted in enhanced selective PL-uptake from HDL. These findings indicate that lipoprotein-associated PL are taken up via SR-BI mediated selective uptake. The present data suggest that SR-BI and EL could be a part of an elaborate system involved in PL-uptake and PUFA generation at the BBB that does not depend on lipoprotein-particle transcytosis.