PROTEOLYSIS OF THE PERICELLULAR MATRIX – A NOVEL ELEMENT DETERMINING CELL SURVIVAL AND DEATH IN THE PATHOGENESIS OF PLAQUE EROSION AND RUPTURE

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The basic pathology behind acute coronary syndromes relates to erosion and rupture of coronary plaques, which trigger the acute atherothrombotic events. Such events may ensue, when the delicate balance between the intrinsic plaque stabilizing and plaque instabilizing processes becomes disturbed. At the cellular level, a switch from cellular growth to cellular death, notably apoptosis, appears to turn the scales in favour of instability. Since smooth muscle cells and endothelial cells require a proper matrix environment for normal function and survival, the vulnerability of an atherosclerotic plaque depends on the integrity of the pericellular matrix (PCM) of these plaque cells. The infiltrating inflammatory cells of hematopoietic origin, macrophages, T-lymphocytes, and mast cells, secrete a variety of proteases capable of degrading PCM components of intimal smooth muscle cells and endothelial cells. This may be followed by apoptotic death of the 2 types of intimal cells, so providing one mechanistic explanation for the inflammation-dependent plaque weakening and rupture. We found that activated mast cells, by secreting the neutral protease chymase, induce apoptosis of cocultured smooth muscle cells and endothelial cells by degrading the PCM necessary for the anchorage of these adherent cells. Loss of focal adhesion points resulted in loss of cell survival signals, with ensuing apoptotic death of the cells. The protease-induced loss of PCM integrity provides a novel link between inflammation and acute coronary syndromes, and aids in understanding of the role of inflammation in the conversion of a clinically silent plaque into a dangerous and potentially killing plaque.

TYPE I COLLAGEN INDUCES MMP1 IN HUMAN SMC: ROLE OF PRENYLATED PROTEINS

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A critical factor in the clinical sequelae associated with lesions of atherosclerosis, such as myocardial infarction and stroke, is the integrity of interstitial type I collagen present in the fibrous cap. Although several soluble factors are known to play a role in collagen homeostasis, no direct therapy for plaque stabilization is currently available. A new interesting possibility comes from the evidence that collagen receptors, express in smooth muscle cells (SMC), can regulate collagen homeostasis in a negative feedback mechanism by inducing matrix metalloproteinases (MMPs) expression. For instance, activation of $\alpha2\beta1$ integrin in human SMC leads to the upregulation of MMP1. In the present study we tested the hypothesis that inhibition of the mevalonate (MVA) pathway and/or function of prenylated proteins might regulate $\alpha2\beta1$ integrin-dependent MMP1 expression. To induce $\alpha2\beta1$ integrin activation we cultured human SMC on polymerized collagen and show that incubation with simvastatin reduced MMP1 levels in both conditioned media (IC_{50}=1.98μM) and cell lysates (IC_{50}=2.05μM). This inhibitory effect seems to lay at the transcriptional level, in fact, reduced MMP1 promoter activity was observed in human SMC incubated with simvastatin. Such effects correlated with a significant reduction of collagen degradation (-34% at 3μM) . Interestingly, MVA, farnesol and geranylgeraniol, almost completely rescue the inhibitory effect of simvastatin, and farnesyl-transferase (FTI-276) and geranylgeranyl transferase inhibitors (GGTI-286) reduced MMP1 upregulation in a dose dependent manner. Taken together, our data demonstrated that inhibition of protein prenylation affects $\alpha2\beta1$ integrin-dependent induction of MMP1 in human SMC. Future studies will be carried to investigate the role of Rho, Rac and Cdc42 on $\alpha2\beta1$ integrin activity.

TUMOR NECROSIS FACTOR-α PROMOTES ATHEROSCLEROTIC LESION PROGRESSION IN APOE*3-LEIDEN TRANSGENIC MICE

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Tumor necrosis factor-α (TNFα) is a pleiotropic cytokine exerting both inflammation and cell death modulatory activity, and is thought to play a role in the pathogenesis of atherosclerosis. Studies in (recombinant) mice indicate that TNFα under conditions that allow fatty streak formation affects atherosclerosis minimally or not. Here, we examined the role TNFα in atherosclerosis under conditions that allow advanced and complex lesion formation.
TNFα-deficient (*Tnf-/-*) mice were combined with atherosclerosis-susceptible APOE*3-Leiden transgenic mice. Feeding APOE*3-Leiden*Tnf-/-* and control APOE*3-Leiden*Tnf*+/+ a western type diet induced hypercholesterolemia (20 mmol/L) that was not affected by the TNFα status. APOE*3-Leiden*Tnf-/-* mice displayed a higher number of circulating T cells (3.2±1.0 vs 1.8±0.6x10^6 CD3+ve cells/ml for control; P=0.01), while the numbers of circulating B-cells (CD19+ve) and monocytes/granulocytes (CD11b+ve) were not affected. In addition, serum amyloid A and plasma soluble intercellular adhesion molecule-1 levels were not affected by the TNFα status. After 20 weeks of diet, both APOE*3-Leiden*Tnf-/-* and control mice were analyzed for atherosclerosis at the aortic root. Although, TNFα absence did not affect the quantitative area of atherosclerosis, APOE*3-Leiden*Tnf-/-* mice had a lower number of advanced (type III-V) lesions as compared to controls (53.9 vs 78.6%, P<0.04). In addition, the advanced lesions in APOE*3-Leiden*Tnf-/-* mice showed significantly less lesion necrosis as compared to advanced lesions in control mice (9.9±12.1 vs 23.4±19.3% of area, P=0.04). Decreased necrosis in advanced lesions of APOE*3-Leiden*Tnf-/-* mice coincided with an increase in apoptosis (1.5±1.5 vs 0.4±0.6% of total nuclei, P=0.03). TNFα-status did not affect lesional macrophage area, smooth muscle cell area or T-cell content.
Our data indicate that TNFα is involved in atherosclerosis by modulating lesional necrosis and the progression of towards a more advanced phenotype.
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SYNERGISTIC ANTI-ATHEROSCLEROTIC EFFECT OF AMLODIPINE AND ATORVASTATIN IN APOE*3-Leiden/hCRP TRANSGENIC MICE

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Introduction The inflammatory marker C-reactive protein (CRP) is a strong predictor of cardiovascular events and statins decrease plasma CRP levels in humans. The direct anti-atherosclerotic effects of calcium-antagonists (CAs) remain under debate. We hypothesized that CAs may have an anti-atherosclerotic effect only when used together with a statin. Therefore, we investigated the effect of the combination of atorvastatin (ator) and amlodipine (amlo) on early atherosclerosis development in APOE*3-Leiden (E3L)/hCRP transgenic mice.
Methods Four groups of 14 male E3L/hCRP mice were fed a cholesterol (chol)-containing diet. One group received 4 mg/kg body weight ator, mixed into the diet, one group 3.5 mg/kg amlo and one group both drugs. The fourth group was the control group (HC). To investigate potential anti-atherosclerotic effects of ator that were not due to lipid lowering, a fifth group (LC) was given a low chol diet that resulted in chol levels comparable to the ator-treated group. After 31 weeks, the mice were sacrificed and atherosclerosis was analyzed in the aortic root area.
Results Plasma chol levels in the HC group were 17 ± 6 mM. Addition of amlo resulted in chol levels of 16 ± 6 mM (n.s.). Ator lowered chol to 10 ± 2 mM (-41%, $p < 0.01$) and combined treatment to 9 ± 2 mM ($p < 0.01$). In the LC group, plasma chol was titrated to 11 ± 4 mM. Amlo did not lower blood pressure as anticipated based on the chosen dosage.
Atherosclerotic lesion area was 26845 ± 16068 μm^2 in the HC group, mainly consisting of type I to III lesions. Treatment with ator resulted in an 80% reduction of lesion area ($p < 0.001$), treatment with amlo in a 43% reduction ($p < 0.03$). Combined treatment decreased the lesion area by 93%, which was significantly more than either treatment alone ($p < 0.008$). The lesion area in the ator group was 30% lower than in the LC control group (n.s.). CRP levels were mildly elevated, on average 10 ± 6 mg/L, and did not differ between groups.
Conclusion This study demonstrates that monotherapy with amlo, independent of blood pressure lowering, or ator reduced early atherosclerosis development in E3L/hCRP mice. Co-treatment with amlo significantly and synergistically enhanced the effect of ator. We did not demonstrate a chol-independent anti-atherosclerotic effect of ator.

ROSUVASTATIN ENHANCES ARTERIOGENESIS IN A MURINE DISEASE MODEL OF APOE-/- DEFICIENCY

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Recruitment, proliferation and growth of pre-existent arteriolar anastomoses to functional collateral arteries (arteriogenesis) serves as an efficient mechanism to prevent devastating consequences of atherosclerotic vessel occlusion. Current pharmaceutical treatment options for atherosclerosis focus on improving the lipid profile by lowering LDL-cholesterol levels through HMG-CoA reductase inhibitors (statins). Since both, arteriogenesis and cholesterol-lowering act together in the improvement of the clinical outcome, we tested the effect of rosuvastatin, the most effective statin to date, on arteriogenesis. **Methods:** Seventy-two ApoE-/- mice received either PBS (control) or rosuvastatin (10mg/kg) subcutaneously once daily after ligation of the right femoral artery. Following seven days or six months of rosuvastatin treatment, hind limb perfusion was assessed using fluorescent microspheres. Blood was collected for evaluation of circulating endothelial progenitor cells (EPC). Histological tissue specimens of the hind limb and the aortic arch were taken and atherosclerotic plaque area of the aorta was quantified. **Results:** Rosuvastatin treatment significantly decreased the aortic atherosclerotic plaque area and improved plaque composition. The amount of circulating EPC was not affected by the treatment. Hind limb perfusion was significantly enhanced by rosuvastatin seven days after femoral ligation (PBS: 26.6 ± 5.3%, rosuvastatin: 35.3 ± 3.7 % of normal; $p<0.001$) and retained a greater level of improvement after six months (PBS: 49.1 ± 6.1%, rosuvastatin: 56.0 ± 5.6 %; $p<0.05$). We have previously demonstrated the importance of monocyte-induced arteriogenesis at the sites of vascular ischemic damage. Quantitative immunohistology revealed an improved migratory ability of monocytes to the site of collateral artery growth. **Conclusion:** Rosuvastatin positively modulates arteriogenesis in atherosclerotic ApoE-/- mice, in the absence of mobilization of EPC. Rosuvastatin also reduced the atherosclerotic burden in these mice.

CAROTID EXTERNAL B-MODE ULTRASOUND VERSUS CORONARY QUANTITATIVE ANGIOGRAPHY AND CORONARY INTRAVASCULAR ULTRASOUND

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Intima-media thickness of extracranic carotid arteries has been proposed to represent a surrogate index of coronary atherosclerosis and this relationship can be derived also from autoptic studies. In contrast, carotid IMT values show only a weak correlation with coronary angiography data ($r<0.30$ always). This apparent discrepancy may derive from the fact that while carotid IMT, determined with B mode ultrasound, is a measure of the size of the arterial wall (IMTs or plaques size), angiographic data are a measure of residual arterial lumen diameter.

Intravascular ultrasound (IVUS), instead, allows the direct examination of coronary vessel wall in living humans, thus providing an in vivo index of coronary atherosclerosis. Therefore, in the present study we investigated the correlation between carotid and coronary atherosclerosis by measuring carotid IMT with coronary atherosclerosis assessed with conventional quantitative angiography and with IVUS. For the preliminary analyses here presented, 32 patients subjected to IVUS for diagnostic reasons were recruited. Spearman correlations between carotid ultrasound measurements and coronary variables measured with quantitative angiography or intravascular ultrasound are listed below.

	Max Stenosis % (quantitative angiography)		**IMT_{Max}** (intravascular ultrasound)	
	r	**p**	**r**	**p**
CC-IMT_{mean}	0.22	ns	0.52	0.008
Bulb-IMT_{mean}	0.33	ns	0.42	0.039
ICA-IMT_{mean}	-0.07	ns	0.03	ns
IMT_{mean}	0.22	ns	0.43	0.033

Thus, carotid IMT significantly correlates with coronary atherosclerosis when is assessed with intravascular ultrasound.

S447X LIPOPROTEIN LIPASE MUTATION IS ASSOCIATED WITH A REDUCTION OF CAROTID INTIMA-MEDIA THICKNESS AND WITH AN IMPROVEMENT OF TRIGLYCERIDES METABOLISM

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Among the enzymes involved in lipoprotein metabolism, LPL plays a central role in controlling the levels of atherogenic TG-rich lipoproteins.

Purpose of this study was to investigate the role of LPL S447X polymorphism, that codes for a protein with an increased enzymatic activity, on development of carotid thickening. We evaluated non invasively intima-media thickness (IMT) of the right carotid artery (CA), as surrogate index of atherosclerosis in almost 2000 individuals in the Northern Area of Milan. We excluded from the data base subjects with CVD, liver or kidney diseases or thyroid dysfunction, and on hypolipidemic or antihypertensive therapies (n=1767) and we selected subjects in the upper RCV% quintile (n=351). Characteristics of these groups are reported in table1. No differences in plasma lipids and lipoproteins were detected between groups.

Table 1 *group*	All subjects	5th quintile RCV	S447X -/-	S447X +/-
n	1767	351	273	78
Age (y)	54±11	62±8	62±7	62±7
RCV % (Framingham)	9.2±7.5	21.4±5.8	21.1±5.7	22.4±6.0
IMT (mm)	0.643 ±0.165	0.736 ±0.194	0.832 ±0.232	0.788 ±0.167*

Values are means ± std error (* $p<0.05$, paired t test).

IMT value was significantly lower in S447X eterozygotes. Absence of differences in the fasting lipoprotein profile led us to investigate whether post-prandial state was affected by the LPL S447X mutation in 25 subjects, age 52 ± 11 years, TC 206 ± 33mg/dL, HDL-C 51 ± 14mg/dL, TG 112 ± 39 mg/dL). Oral fat load results are reported in table2.

Table 2 *group*	*n*	*AUC Total TG (mg/dL)*	*AUC TGRL (mg/dL)*	*AUC RLP Tg (mg/dL)*
S447X -/-	19	1423 ± 126	1141 ± 112	647 ± 66
S447X +/-	6	995 ± 206	774 ± 178	407 ± 30*

Values are means ± std error. (*p=0.008, paired t test)

Lower levels of plasma and remnant lipoprotein TG in presence of the S447X mutation may explain the reduced IMT in subjects with this polymorphism.

REGULATION OF APOLIPOPROTEIN B METABOLISM IN THE METABOLIC SYNDROME

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Metabolic syndrome is an insulin resistant, chronic inflammatory state associated by definition with central obesity, dyslipidemia and moderate hypertension. The main lipid abnormality present has been termed the atherogenic lipoprotein phenotype (ALP) or "lipid triad" and is characterised by elevated plasma triglyceride, low HDL cholesterol and a predominance of small, dense LDL. These lipoprotein changes are a key component of the CHD risk associated with the syndrome.

Perturbations in VLDL and triglyceride metabolism are central to the generation of an ALP. Accumulation of visceral adipose tissue leads to excessive supply of fatty acid to the liver and in centrally obese subjects VLDL is overproduced (Chan et al Metabolism 2002;51:1041-6). Insulin resistance leads also to a failure to suppress production of large triglyceride rich VLDL during feeding. The consequent hypertriglyceridemia generates changes in LDL and HDL structure and metabolism, via neutral lipid exchange and lipolysis. Hepatic lipase, cholesteryl ester transfer protein activity and the residence time of LDL particles are all regulatory factors that determine the extent to which an ALP develops in the presence of hypertriglyceridemia.

Investigation of the actions of diet and drugs on apolipoprotein B metabolism in metabolic syndrome has shown that the dyslipidemia is amenable to correction through a number of interventions. Weight loss with reduction in visceral fat is associated with decreased VLDL production while statin and fibrates act mainly by accelerating clearance of apoB containing particles from the circulation. Recently we have shown that on statin therapy, plasma levels of VLDL, IDL and LDL are decreased in concert as a result of increased catabolism. The fall in small, dense LDL and remnant particles was observed to be related to the magnitude of decrease in large VLDL. There is increasing evidence that statins have both phenotype-independent and phenotype-dependent (related to plasma triglyceride) actions (Caslake et al Atherosclerosis 2003 171;245-53).

ENDOTHELIAL LIPASE AS A POTENTIAL PHARMACOLOGIC TARGET FOR HDL AND ATHEROSCLEROSIS

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Endothelial lipase (EL) is member of the triacylglycerol lipase gene family that includes LPL and HL. EL has considerably greater phospholipase activity and greater ability to hydrolyze HDL compared with LPL and HL. The C-terminus plays a critical role in determining the ability of EL to bind to and hydrolyze HDL. Overexpression and loss-of-function studies in mice indicate that EL plays an important role in HDL metabolism. Endothelial expression of EL is upregulated by cytokines. EL binds to heparin sulfate proteoglycans and is released by heparin. Administration of heparin to humans results in a 4-fold increase in plasma levels of EL. Both pre- and post-heparin plasma levels of EL in humans are inversely associated with HDL cholesterol levels and directly associated with coronary atherosclerosis. Thus, EL is a potential pharmacologic target for inhibition with the goal of raising HDL-C levels and reducing atherosclerosis.

TRIGLYCERIDES RICH-LIPOPROTEINS FROM HYPERTRIGLYCERIDEMIC SUBJECTS INDUCE A PRO-INFLAMMATORY PATTERN IN ENDOTHELIAL CELLS: MOLECULAR MECHANISMS AND GENE EXPRESSION STUDIES.

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Objective: Elevated levels of triglyceride-rich lipoproteins (TGRL) are a cardiovascular risk factor and have been shown to induce endothelial dysfunction; however the molecular mechanisms and the gene expression patterns underlying these effects are poorly understood. In the present work we investigated the effects of triglyceride-rich lipoproteins from hypertriglyceridemic and normotriglyceridemic subjects on endothelial function focussing on activation of intracellular pathways and gene expression in human endothelial cells.
Results: A total of 53 subjects, 30 hypertriglyceridemic (TG levels 284,4 ±101,1 mg/dL) and 23 normotriglyceridemic (TG levels 108,65 ±39,9 mg/dL) were enrolled into the study. Human endothelial cells were incubated with TGRL isolated from hypertriglyceridemic (H-TGRL) and normotriglyceridemic (N-TGRL) subjects. Western blotting analysis and protein/DNA arrays showed that H-TGRL mainly activated p38MAPK, CREB and NF-kB, AP-1 as well as Bm-3, CDP, NF-1, NFE1, NFE2. Total RNA was processed for cDNA microarray analysis. H-TGRL mainly induced the expression of adhesion molecules such as VCAM-1, PECAM-1, ELAM-1, P-selectin, chemotactic factors such as MCP-1, cytokines such as IL-6, receptors such as TLR-4 and CD40, and proteases such as PAI-1 and ADAMTs1. This profile was characteristic of H-TGRL as N-TGRL increased only the expression of VCAM-1, PECAM-1 and PAI-1. These findings were validated with quantitative real-time PCR and immunofluorescence studies. Furthermore, bioinformatic analysis and chromatin-immunoprecipitation (CHIP) studies confirmed that NF-kB and CREB were mainly responsible for the effects observed.
Conclusion: These findings confirm the involvement of TGRL from hypertriglyceridemic subjects in endothelial dysfunction via the induction of pro-inflammatory and pro-thrombotic responses.

LDL ISOLATED FROM SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE INCREASE THE EXPRESSION OF CD36 AND PPARγ IN MACROPHAGES

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Subjects with impaired glucose tolerance (IGT) had significantly increased serum levels of oxidized LDL, indicating a link between oxidative stress and atherosclerosis. We investigated the effects of LDL isolated from 20 IGT subjects (IGT-LDL) and a control group with normal glucose tolerance (NGT-LDL, n=20) on the expression of the class B scavenger receptors CD36 and SR-BI, and the transcriptional regulator PPARγ in murine macrophages (cell line RAW 264.7).
Macrophages were treated for one hour with LDL (30 µg/mL protein concentration). After incubation, mRNA was extracted and converted to cDNA with subsequent quantitative analysis by real-time PCR. Additionally, biochemical composition of LDL particles was analysed.
Incubation of mouse macrophages with IGT-LDL resulted in significant higher gene expression of CD36 and PPARγ when compared with LDL from NGT controls ($p<0.05$ by ANOVA). No differences of LDL effects on SR-BI gene expression could be detected. PPARγ gene expression did show a strong negative correlation with MCP-1 cytokine levels in supernatant of macrophages after exposure to IGT-LDL ($r=0.699$, $p=0.003$), but not to NGT-LDL.
In conclusion, the enhanced CD36 and PPARγ expression induced by IGT-LDL as compared to NGT-DL suggests that already in prediabetic states LDL exhibit physico-chemical modifications with proatherogenic relevance. The observed negative correlation between PPARγ expression and MCP-1 release from macrophages stimulated with IGT-LDL points towards the dual role of PPARγ as a proinflammatory and antiinflammatory transcriptional regulator.

ASSOCIATION BETWEEN MARKERS OF INFLAMMATION AND LOW HDL CHOLESTEROL LEVELS. RESULTS FROM THE InCHIANTI STUDY

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Low HDL-C levels are a risk factor for CHD. Besides genetics, other factors influence HDL levels such as body composition, life style, and alterations of lipid/glucose metabolism. A growing level of attention has been devoted to the relationship between inflammation and lipoproteins. In this study we investigated the association between markers of inflammation and low HDL-C by analyzing data from the InChianti Study, a large sample of Italian subjects, including 1270 older subjects. Low HDL-C was defined ≤10th percentile (36 mg/dL M, 41 mg/dL F). Acute phase marker considered were: IL-6, IL-10, IL-18, IL-1β, TNFα, and CRP. HDL-C levels were measured by the Trinder method. Only IL-6, IL-18 e CRP were inversely correlated with HDL-C (adjusted R^2:0.12). BMI, waist and hip circumferences, triglycerides (TG), glucose, white blood cells count, haemoglobin, and smoking were also inversely correlated with HDL-C. High levels of IL-6 were more frequent among subjects with higher BMI, waist circumference, fasting insulin, and TG (χ^2 test p: 0.001). Logistic regression analysis showed that IL-6 levels (III vs I tertile, OR:2.01; 1.08-3.70), TG (III vs I tertile O.R.: 27.49; 8.48-89.01), fasting insulin (III vs I tertile O.R.:2.83; 1.49-5.39), and age (O.R.:1.03; 1.001-1.07) were associated with low HDL-C levels independent of gender, smoking, alcohol intake, diabetes, BMI, waist circumference, IL-18, IL-1β, IL-10, and CRP levels. We conclude that: 1) some markers of inflammation are associated with HDL-C levels in older individuals; 2) at multivariate analysis only IL-6 levels are associated with low HDL-C values; 3) although IL-6 levels were associated with the main traits of the metabolic syndrome its association with low HDL-C is independent.

PROTEOLYSIS OF PLASMA HDL PARTICLES BY MAST CELL NEUTRAL PROTEASES: MULTIPLE EFFECTS ON HDL FUNCTIONS IN REVERSE CHOLESTEROL TRANSPORT

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Mast cells in the human arterial intima are filled with neutral proteases, either tryptase alone or in combination with chymase. Upon activation, the mast cells secrete these proteases, and, in the extracellular fluid, they remain partially active. We found that limited proteolysis of HDL_3 by human chymase and human tryptase specifically cleaves the minor prebeta-HDL particle fraction, whereas the integrity of major alpha-HDL fraction remains largely unaltered. These proteolytic modifications of HDL blocked the high-affinity component of cholesterol efflux from macrophage foam cells *in vitro*, reflecting that intactness of apoA-I in prebeta-HDL is crucial for promotion of this process. The high-affinity component of cholesterol efflux was found to be ABCA1-mediated. In contrast, chymase treatment did not influence the aqueous or SR-BI-facilitated diffusional pathways of cellular cholesterol efflux. In other experiments, we found that chymase treatment of HDL_3 increases the ability of apoA-I to interact with the 2 plasma lipid transfer proteins PLTP and CETP. Overall, the results reveal multiple effects on HDL functions in reverse cholesterol transport (RCT). In terms of the very initial steps of RCT, the results demonstrate that the cholesterol efflux component promoted by prebeta-HDL is protease-sensitive, but that the other component mediated by alpha-HDL is not protease-sensitive. The results also reveal that the high-affinity cholesterol efflux from macrophage foam cells can be blocked, in addition to the known genetic and metabolic intrinsic factors affecting the activity of the ABCA-1 transporter, also by extrinsic proteolytic factors which incapacitate the acceptors of this pathway. We conclude that, by blocking the initiation of reverse cholesterol transport, the protease-secreting mast cells can potentially induce formation and maintenance of macrophage foam cells in atherosclerotic lesions.

PPARδ REGULATES VLDL PRODUCTION AND CATABOLISM IN MICE ON A WESTERN DIET

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The results of recent studies using selective agonists for PPARδ suggest that this receptor may have a role in regulating levels of serum lipids in animal models of obesity and insulin resistance. To further examine this possibility, serum lipid profiles of mice lacking a functional PPARδ receptor were determined. PPARδ-null mice maintained on either normal chow or a 10-week high fat (HF) diet, a condition that has been shown to induce insulin resistance and obesity in mice, have elevated levels of serum triglycerides primarily associated with very low density lipoprotein (VLDL) with no difference in either total cholesterol or phospholipids. Consistent with this finding, PPARδ-null mice on a HF-diet were shown to have an increased rate of hepatic VLDL production as well as lowered lipoprotein lipase activity in serum compared to wild-type controls. The latter parallels an increase in the hepatic expression of the genes encoding angiopoietin-like proteins 3 and 4 in PPARδ-null mice on a HF diet, both proteins of which have recently been shown to inhibit lipoprotein lipase (LPL) activity in vivo. Consistent with elevated VLDL production, a marked increase in plasma VLDL apoB48, E, AI, and AII was also found in PPARδ-null mice. In addition, PPARδ-null mice on a HF diet were shown to have increased adiposity, despite lower total body weight. Together, these results indicate a clear role for PPARδ in regulating levels of serum triglycerides in mice on a high fat western diet by modulating both VLDL production and LPL-mediated catabolism of VLDL-triglycerides, and also suggest a potential therapeutic role for PPARδ in the improvement of serum lipids in the setting of metabolic syndrome.

PATHOPHYSIOLOGY OF THE ATHEROSCLEROTIC PLAQUE

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Not so long ago most viewed atherosclerosis as a bland deposit of lipid clogging arteries. Our understanding of the biology of this disease has burgeoned recently. These investigations have pinpointed inflammation as a central process in all stages of atherosclerosis. The formation of the early lesions of atherosclerosis involves the recruitment of mononuclear leukocytes including monocytes and T lymphocytes. Pro-inflammatory cytokines such as interleukin-1, tumor necrosis factor, and CD40 ligand (CD154) can induce the expression of adhesion molecules on the endothelial cell surface that bind circulating white blood cells at sites of local arterial inflammation. Chemokines such as MCP-1 direct the transendothelial migration of these adherent monocytes. Indeed, interruption of chemokine signaling inhibits formation of experimental atheroma. Other protein mediators, the colony stimulating factors, such as M-CSF and GM-CSF, also activate macrophages during atherogenesis. Later, leukocytes now resident in the forming lesion may themselves elaborate cytokines, amplifying the regional inflammatory response in the artery wall. Inflammation also regulates the progression of atherosclerosis. Among the products elaborated by macrophages exposed to inflammatory cytokines, growth factors (including platelet-derived and fibroblast growth factor family members) can beckon smooth muscle cells (SMC) to enter the intima from the underlying tunica media, stimulate their proliferation, and manufacture of extracellular matrix proteins that render the fatty plaque more fibrous. Inflammatory mediators such as CD40 ligand can promote the progression of lesions in experimental atherosclerosis. Oxidant stress due to enzymes such as NAD(P)H oxidases and myeloperoxidases can contribute to inflammation and lesion evolution. Once established, lesions lead to acute clinical complications because of thrombosis. We have gained considerable insight into the mechanisms of these processes, based on the regulation of the strength of the collagen that protects the plaque from rupture and the factors that govern the plaques thrombotic potential. Recent work in genetically-altered mice demonstrate that collagen breakdown by matrix metalloproteinases (MMPs) contributes to collagen accumulation in atheromata. Inflammatory mediators govern these mechanisms that underlie the dreaded thrombotic complications, the ultimate expression of this disease. Thus, from the earliest steps of lesion formation, through the usually prolonged phase of silent or stable progression, up to the acutely occluding thrombus, inflammation orchestrates the complex biology of atherosclerosis.

DIABETES AND ATHEROSCLEROSIS; NOVEL POTENTIAL TARGETS FOR THERAPY

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In Type 2 diabetes dyslipidemia is an important and common risk factor for coronary heart disease (CHD) that is the leading cause of morbidity and mortality worldwide. The benefits of statin therapy in people with Type 2 diabetes are indisputable but statin therapy is not able to wipe out the risk linked to low HDL cholesterol. Diabetic dyslipidemia is a cluster of atherogenic components including small dense LDL, cholesterol-ester rich remnants and low concentration of HDL particles with unfavorable compositional alterations. The coexistence of these three factors strongly aggravates the lipid accumulation in the arterial wall and should be the targets of therapy. Growing evidence suggest that the increase of large VLDL1 particles is the culprit of diabetic dyslipidemia and the unifying factor behind the atherogenic components of diabetic dyslipidemia. Dysregulation of fat metabolism in the liver seems to be the factor leading to the overproduction of large VLDL1 particles in type 2 diabetes Thus the regulatory steps in VLDL assembly come up as novel targets for drug action. The machinery driving VLDL assembly in the liver is complex and not well established. The key question is how liver is able to regulate the amount of triglycerides incorporated into VLDL particles to produce predominantly either large VLDL1 particles or smaller VLDL2 particles? Potential factors that modulate VLDL assembly in the liver include increased FFA flux into the liver, impaired insulin signalling via PI-3 kinase pathway that enhances lipid accumulation into "nascent" VLDL particles, excess availability of "fat" in the hepatocytes that stabilizes apo B and up-regulation of SREBP-1C that drives de novo lipogenesis. Other transcription factors like PPARs and LXRs also are key players in the regulation of fat metabolism in the liver. Growing evidence suggest that all these steps are pertubated in Type 2 diabetes and in insulin resistance. These factors provide new targets to tailor novel drugs that specifically capture the initial sites of VLDL assembly.

CARDIOVASCULAR PROTECTION BY ANTIHYPERTENSIVE TREATMENT: EVIDENCE FROM CONTROLLED TRIALS

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Hypertension is a major risk factor for cardiovascular disease. Evidence from a large number of controlled trials, however, shows that this risk is not irreversible but that a blood pressure reduction obtained via drug treatment markedly reduces the cardiovascular risk. This presentation will review the evidence that 1) cardiovascular protection can be achieved by a variety of antihypertensive drugs 2) in a number of conditions (e.g. diabetes and very high risk hypertensives) aggressive blood pressure reductions enhance the protective effect 3) this can be obtained also by associating effective antihypertensive treatment with treatments of other risk factors common in hypertension, e.g. hypercholesterolemia and 4) there is some evidence that for a given blood pressure reduction some antihypertensive drugs may have greater specific organ protective properties (kidney and brain) as compared to others. There is also evidence that some antihypertensive drugs are more effective in protecting patients against new onset diabetes which may have relevance for control of total cardiovascular risk and long-term protection. This presentation will finally show that in the majority of hypertensive population blood pressure control remains an elusive goal, with serious complications for public health.

ATHEROSCLEROSIS AND THE METABOLIC SYNDROME

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The major risk factors for atherosclerotic cardiovascular disease (ASCVD) are cigarette smoking, hypertension, elevated cholesterol, low HDL cholesterol, diabetes mellitus, and advancing age. All of these risk factors have been shown to be accompanied by increases in atherosclerotic disease of the coronary arteries and/or aorta. In addition, as a consequence of the growing prevalence of obesity and sedentary life habits world wide, a new multidimensional risk factor, the metabolic syndrome, is emerging. This "risk factor" includes five different metabolic risk factors: atherogenic dyslipidemia (elevated apolipoprotein B and triglycerides, small LDL particles, and low HDL), elevated blood pressure, elevated glucose, a prothrombotic state, and a proinflammatory state. There are several underlying risk factors for the metabolic syndrome: obesity, insulin resistance, physical inactivity, and hormonal imbalance. Although some of the metabolic risk factors are included among the major risk factors for ASCVD, they are often present at levels that are often not considered to be categorical risk factors. However, the accumulation of multiple marginal risk factors combined produces a risk at least equivalent to that of a major risk factor. Several reports now show that persons with the metabolic syndrome are at much higher risk for ASCVD than those without metabolic syndrome. In addition, once the plasma glucose level reaches that diagnostic of type 2 diabetes, risk increases even more. Indeed, world wide, much of the growing prevalence for ASCVD can be explained by an increased prevalence of metabolic syndrome that has advanced to type 2 diabetes.

PERSPECTIVES ON STATINS IN CARDIOVASCULAR MEDICINE AND INFLAMMATORY DISEASES

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Lipid-modifying therapy with the 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors, or statins, have emerged at the forefront of pharmacologic strategies for cardiovascular risk reduction. Recent clinical trial data have broadened substantially the kinds of patients who might be considered for statin treatment. Increasingly, scientists acknowledge the role of chronic inflammation in atherogenesis. Recent studies have ignited increased scrutiny of the non-lipid effects of statins, most notably their potential effects on inflammation. Statins are known to decrease levels of C-reactive protein, an inflammatory marker with novel cardiovascular risk assessment potential, and may affect the balance of immune response in vivo. Based on preliminary small studies, investigators are currently examining the effect of statins in neuroinflammatory disorders, such as multiple sclerosis. Considering the effects of statins beyond the coronary arteries as well as optimizing the appropriate use of statin treatment in cardiovascular medicine are exciting challenges for the coming years. The results of exploring these areas may provide new insights into the etiology of atherosclerotic disease and identify new approaches to intervention.

THE ENDOCANNABINOID SYSTEM: NEW THERAPEUTIC TARGET FOR ENERGY BALANCE AT BOTH CENTRAL AND PERIPHERAL LEVELS

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Endocannabinoids (ECBs) and in particular anandamide and 2-arachidonoylglycerol are endogenous lipids capable of binding to, and subsequently activating, the 2 cannabinoid (CB) receptors CB_1 and CB_2. These receptors belong to the G-protein–coupled family receptors and they were discovered in the early 1990s while investigating the molecular mode of action of the principal psychoactive component of *Cannabis*, Δ^9-tetrahydrocannabinol, to which they bind with high affinity. CB_1 is the most widespread CB receptor in mammalian tissues, with the highest concentrations in some brain areas, but also present in many other peripheral organs, including the adipose tissue, gastrointestinal system, the airways, the reproductive organs, and the cardiovascular system. Stimulation of the CB_1-receptor by its agonists, including the ECBs, leads to inhibition of neurotransmitter release in central and peripheral (both autonomic and sensory) neurons. In non-neurons, several functions have been associated with CB_1 stimulation, including the regulation of proliferation, differentiation, motility, and apoptosis, possibly through modulation of the expression of various growth factors. ECBs are biosynthesized subsequent to the Ca^{2+}-dependent remodeling of membrane phospholipids, followed by the enzymatic hydrolysis of specific lipid precursors. This means that ECBs are not stored in neurons prior to their release, but are rather released "on demand" immediately after their *de novo* biosynthesis. In other words, the basal levels of ECBs are barely detectable as they are produced only "when and where needed," to be then rapidly inactivated by hydrolytic enzymes. Regarding their possible biologic function, the general picture emerging from studies carried out during the last 10 years is that ECBs are produced, and CB_1receptors stimulated, in response to stressful stimuli to help establish the steady state homeostasis of other neurotransmitters, mediators, hormones, and cytokines. Therefore, CB_1-receptor stimulation is short-lasting, limited to those cells or tissues that have been subjected to stress or damage, and normally ends once the organism has recovered from a transient "unbalanced" condition. However, some chronic pathologic states lead to long-lasting overstimulation of ECB synthesis (or hypo-stimulation of their degradation), resulting in permanent overactivation of CB_1 receptors, which may then contribute to the symptoms of these disorders. The ECB system is present in brain and peripheral sites involved in the control of energy balance and body weight, as well as in neurons of the mesolimbic system that participate in reinforcing reward and in "translating motivation into action". At central nervous system CB_1-receptors are necessary to induce food intake after a short period of food deprivation and when activated they also preferentially stimulate the ingestion of palatable food. Their stimulation leads to modulation of the release and/or expression of some hypothalamic anorexigenic and orexigenic mediators, as well as of dopamine in the nucleus accumbens shell. Importantly, at peripheral level CB1 activation has been shown to stimulate lipogenesis into adipocytes, and CB1 blockers are important positive modulators of adiponectin secretion. These physiological properties of ECB and CB1, the pathologic consequences of an altered cannabinoid tone, and its pharmacological management will be discussed in this presentation.

CB1 BLOCKADE FOR THE MANAGEMENT OF METABOLIC RISK DISORDERS

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Rimonabant (R) is the first selective cannabinoid type 1 (CB_1) blocker developed for the treatment of obesity and smoking cessation. RIO-Lipids is a multinational, multicenter, randomized, double-blind, placebo-controlled, one-year treatment study. Main inclusion criteria were 27< body mass index (BMI) ≤40 kg/m^2, untreated dyslipidemia with 1.69 ≤ triglycerides (TG) ≤ 7.90 mmol/L and/or total cholesterol/HDL-cholesterol (C) ratio >4.5 (female) >5 (male) with fasting plasma glucose <6.99 mmol/L. A total of 1,036 overweight/obese subjects, mean age 47.8 years, mean BMI 34.0 kg/m^2, mean weight 96.1 kg, were randomized to receive placebo, R5mg or R20mg once daily. Primary efficacy end points were weight loss and weight maintenance at 1 year. R20mg induced a significant reduction in both body weight (-6.9 kg) and waist circumference (-7.1 cm), such reductions being significantly greater than in the placebo group ($p<0.001$). Such substantial loss of weight and of abdominal fat induced by R20mg was accompanied by significant improvements in the plasma lipoprotein-lipid profile which included reduction in plasma TG levels (-0.40 mmol/L) and a marked increase in HDL-C concentration (+0.20 mmol/L), both changes being significantly greater than in the placebo group ($p<0.001$). Whereas rimonabant had no significant effect on both cholesterol and LDL-C levels, the distribution of LDL particle sizes was altered as a decrease in the proportion of atherogenic small LDL (-4.7%, $p=0.002$) and an increase in the percentage of large LDL particles (+6.3%, $p<0.001$) were observed with R20mg therapy. As compared to placebo, R20mg induced significant improvements in the overall plasma glucose and insulin responses to the oral glucose load ($p<0.001$). Prevalence of RIO-Lipids patients meeting the NCEP-ATP III criteria for the presence of the metabolic syndrome revealed that it was reduced from 52.9% to 25.8% among patients treated with R20mg. R5mg results were either similar to those of placebo or intermediate to those of placebo and R20mg. Rimonabant was well tolerated. Overall, these results suggest that rimonabant therapy could be useful for the management of clustering cardiovascular disease risk factors in high-risk abdominally obese patients through its marked effects on both abdominal adiposity and related metabolic risk factors.

RATIONALE FOR TARGETING MULTIPLE LIPID PATHWAYS FOR OPTIMAL CARDIOVASCULAR RISK REDUCTION

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Although clinical trials of statin therapy have demonstrated large reductions in low-density lipoprotein cholesterol (LDL-C) and significant reductions in risk for coronary artery disease (CAD) events in both primary and secondary prevention, patients treated with statin monotherapy have a residual CAD risk that may be attributable to abnormalities in other lipid parameters or to a failure to reduce LDL-C to optimal therapeutic goals. Combining a statin, which inhibits cholesterol synthesis, with agents with complementary mechanisms may provide greater improvements for the entire lipid profile and greater benefits clinically for atherothrombotic disease. In patients with mixed dyslipidemia, combination therapy with a fibrate, niacin, or another agent may be required to normalize high-density lipoprotein cholesterol (HDL-C) and triglycerides. Patients whose LDL-C is not adequately reduced with statin monotherapy may require the addition of an agent that inhibits a different pathway, such as a bile acid resin, niacin, or a cholesterol absorption inhibitor, a new class of lipid-lowering agents that inhibits intestinal cholesterol absorption. Dual inhibition of cholesterol synthesis and absorption has been shown to provide significantly greater reductions in LDL-C and triglyceride and greater increases in HDL-C than statins alone, and to enable a greater proportion of patients to achieve LDL-C targets. In addition to providing greater lipid effects, combination therapy has been reported to provide benefit on nonlipid parameters. Combining as statin with a cholesterol absorption inhibitor yields a greater reduction in C-reactive protein than statin monotherapy. Large randomized trials will be needed to evaluate incremental additional clinical benefit of combination therapy regimens versus statin monotherapy in different patient populations.

MODIFYING PLASMA LDL AND HDL CHOLESTEROL, WHAT COMBINATIONS ARE AVAILABLE IN THE FUTURE

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Coronary heart disease (CHD) is the primary cause of death in Europe and the United States. High serum concentrations of low-density lipoprotein cholesterol (LDL-C) increase the risk of CHD. Despite published guidelines for assessment and treatment of hypercholesterolemia, many patients are inadequately treated, and many do not reach target LDL-C goals. Statins are the most frequently used drug for treatment of hypercholesterolemia; however, most patients do not achieve target goals with starting statin doses. High doses of statins or statins combined with other lipid-modifying agents may be necessary to reach LDL-C targets, but these strategies are associated with increased risk of side effects and low patient acceptance. New agents that can be used safely alone or in combination with statins to attain target goals are needed. Combining drugs with different pathways of cholesterol metabolism may provide benefits that are complementary and additive to those of the statins. For example, ezetimibe, the first selective cholesterol absorption inhibitor, effectively blocks intestinal absorption of dietary and biliary cholesterol and, when co-administered with a statin, provides an additional 16% to 18% reduction in LDL-C levels. The need for less frequent statin dosage adjustments may lead to improved patient compliance and help more patients attain their LDL-C goals.

Other cholesterol-lowering agents (eg, niacin or nicotinic acid and the fibric acid derivatives [gemfibrozil, fenofibrate, bezafibrate, and clofibrate]) interfere with synthesis and release of fatty acids, VLDL-C, the precursor of LDL-C, reducing both VLDL-C and plasma LDL-C levels, if TG levels are not too high. Since fibrates also increase the activity of lipoprotein lipase, increasing the removal of TG and substantially decreasing plasma TG, fibrates are often first-line drugs for patients with hypertriglyceridaemia. Nicotinic acid effectively increases HDL-C concentrations but causes intolerable flushing of the skin in about 10% of patients. Hepatotoxicity can also occur in patients taking niacin, especially time-release formulas. A new extended-release form of niacin might help reduce flushing while minimising the hepatotoxicity.

Studies of combination therapy with statins and niacin, statins and bile acid sequestrants, and statins and fibrates have all demonstrated that combination therapy is as effective as or superior to either drug given as monotherapy for lipid management.

The most promising combination, however, will have to both effectively reduce LDL-C and increase HDL-C beyond the current possibilities. The latter is feasible now by inhibiting cholesterylester transfer protein C (CETP), leading to HDL-C elevations of 50% and beyond. This lecture will cover the future use of CETP inhibitors and powerful statins in the prevention of CAD.

EMERGING THERAPEUTIC STRATEGIES FOR THE MANAGEMENT OF DYSLIPIDEMIA IN PATIENTS WITH THE METABOLIC SYNDROME

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The benefits of lipid-lowering therapy in significantly reducing cardiovascular events have been established in many at-risk populations. However, patients with the metabolic syndrome (MS) pose a challenge for clinical management. A high degree of residual risk exists in patients with the MS or diabetes and this is of growing importance due to the increasing prevalence of obesity and the associated co-morbidities in the world. Recent information from an analysis of NHANES III data using Framingham risk prediction algorithms to estimate CAD event risk highlights the effect of lipid control in adults who met ATP III criteria for the MS. Data estimates were extrapolated to demonstrate that 1.5 million of 7.5 million men with the MS (20%) and 0.45 million of 9 million women with the MS (5%) in the US would have CAD events > 10 years. Optimal control of LDL cholesterol to <100 mg/dL, HDL cholesterol levels of 60 mg/dL and blood pressure control would prevent 80.5% and 82.1% of events in men and women, respectively. Indeed, treatment of risk factors could decrease the burden of CAD in the MS population. In addition, new scientific evidence suggests that more widespread definition of high-risk patients will significantly impact the need for lipid therapy. These include the use of hs-CRP and the MS as high-risk groups for treatment, new targets for treatment including apoB/apoA-1 ratio, use of a global risk score and revised recommendations for initiation of therapy for high-risk patients. Based on the Heart Protection Study results demonstrating a benefit of lipid-lowering therapy regardless of baseline LDL, the clinical judgement zone at 100 to 130 mg/dL should no longer exist. In fact, therapeutic LDL cholesterol targets may be lowered even more based on several ongoing large scale clinical trials that are evaluating the clinical benefits of an LDL cholesterol target of 70 mg/dL compared with an LDL cholesterol target of 100 mg/dL. As the MS has emerged as a major risk factor for both cardiovascular disease and diabetes, targeting treatment to achieve aggressive goals becomes paramount.

COMBINATION THERAPY FOR CARDIOVASCULAR DISEASE RISK REDUCTION

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Polypharmacy to achieve multiple risk factor modification for cardiovascular disease risk reduction has become standard of care. This is based on hypotheses driven by a growing understanding of pathophysiology and by analyses of epidemiologic databases, and, perhaps more directly, on the primary and non-primary results of large endpoint trials of pharmacologic therapeutics. In patients with significant, identifiable cardiovascular risk, including those with diabetes, metabolic syndrome, and manifest cardiovascular disease, multiple drugs are used to address the panoply of metabolic abnormalities that are believed to mediate, collectively, the atherosclerotic process and its clinical consequences.

The approval of combination therapeutic regimens, whether providing for labeling of marketed component drugs or constituting initial approval and labeling of fixed-dose combination drug products requires foremost that each component of the combination contributes to the overall effect. Combinations of drugs that impact the same "validated" pharmacodynamic endpoint (e.g., blood pressure, blood glucose) may be rationalized based on efficacy as well as safety (dose- and therefore toxicity-sparing). Combinations (specifically fixed combinations) of drugs affecting different endpoints or mediators of CVD (e.g., LDL-C and HDL-C, glucose and blood pressure) though presumably acting additively to reduce CVD are considered "combinations of convenience". Approval of such products requires: 1) the existence of a definable population of patients taking the component drugs, 2) the availability of all or adequate combinations of dosage strengths of component drugs so as not to drive inappropriate use of unsafe or ineffective doses of component drugs, 3) the absence of drug-drug kinetic or dynamic interactions that might adversely affect the efficacy or augment the risk associated with one or another component drug, or engender a novel adverse drug effect, and 4) evidence (e.g., from factorial design clinical trials to surrogate or hard endpoints, from analyses of subgroups of completed trials) to establish presumptive additive benefits of component drugs on outcomes.

LXR AGONIST, R-196379 AND ATORVASTATIN REDUCE ATHEROSCLEROTIC LESION IN A SYNERGISTIC MANNER IN LDL RECEPTOR DEFICIENT MICE

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The nuclear receptors LXRα and LXRβ regulate the expression of genes involved in cholesterol homeostasis, including ATP binding cassette transporter ABCA1. The expression of ABCA1 is crucial for reverse cholesterol transport, the process by which peripheral cells efflux cholesterol to HDL. We have previously demonstrated that LXR agonist inhibited progression of atherosclerosis in LDL receptor $(LDLR)^{-/-}$ mice. The aim of the present study was to determine whether R-196379, a novel LXR agonist, would exert an anti-atherosclerotic effect in $LDLR^{-/-}$ mice. Moreover, atorvastatin, an HMG-CoA reductase inhibitor, was evaluated under the same experimental conditions as well as in combination with R-196379, to determine whether the combination treatment would elicit an additive or synergistic effect. R-196379 at 0.1 and 0.3 mg/kg significantly reduced atherosclerotic lesion without affecting lipid profiles. The anti-atherosclerotic effect revealed a correlation with macrophage ABCA1 expression. In addition, R-196379 reduced the expression of the inflammatory factors. Atorvastatin at 30 mg/kg also reduced atherosclerotic lesion area by 39%. The anti-atherosclerotic effect appears to be due to reduction of plasma lipids. The combination treatment of R-196379 with atorvastatin synergistically reduced the atherosclerotic lesion by 81%, while the magnitude of the lipid lowering effect was equivalent to that of atorvastatin alone. Thus, the synergistic anti-atherosclerotic effect is due to the combination of the different actions of both compounds. Here we provide the first evidence that combination treatment of an LXR agonist with an HMG-CoA reductase inhibitor would be efficacious therapeutic treatment for atherosclerosis.

EFFICACY AND SAFETY OF COADMINISTERED FENOFIBRATE AND EZETIMIBE IN PATIENTS WITH MIXED HYPERLIPIDEMIA

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Coadministration of fenofibrate (FENO) and ezetimibe (EZE) was examined for efficacy and safety in patients with mixed hyperlipidemia. After a 6-8 week washout period, patients with LDL-C 3.4-5.7 mmol/L [2.6-4.7 mmol/L for patients with type 2 diabetes] and triglycerides (TG): 2.3-5.7 mmol/L, and no history of CHD, CHD-equivalent disease (except type 2 diabetes), or CHD risk >20% per NCEP ATP III criteria were randomized in a 1:3:3:3 ratio to 1 of 4 daily treatments for 12 wks: placebo; EZE 10 mg; FENO 160 mg; FENO 160 mg + EZE 10 mg. The primary endpoint compared the LDL-C lowering efficacy of FENO+EZE vs. FENO alone. LDL-C, non-HDL-C, and apo B were significantly reduced with FENO+EZE compared with FENO or EZE. HDL-C was significantly increased and TG significantly reduced with FENO+EZE and FENO. All active therapies were well-tolerated. Coadministration of FENO+EZE provides a complementary efficacy therapy that improves the atherogenic lipid profile of pts with mixed hyperlipidemia.

Mean Percent change	PBO N = 63	EZE N = 185	FENO N = 188	FENO+EZE N = 183
LDL-C	0.2[a]	-13.4[a]	-5.5[a]	-20.4
HDL-C	3.2[a]	3.9[a]	18.8	19.0
TG[#]	-9.2[a]	-11.1[a]	-43.2[b]	-44.0
Non-HDL-C	-0.2[a]	-14.7[a]	-16.2[a]	-30.4
apo B	-1.2[a]	-11.3[a]	-15.2[a]	-26.1

Apo = apolipoprotein; PBO = placebo; [#]median; [a]p<0.001 compared to FENO+EZE; [b]p=0.021 for FENO+EZE vs. FENO

INHIBITION OF CHOLESTERYL ESTER TRANSFER PROTEIN BY JTT-705 IN COMBINATION WITH PRAVASTATIN IN TYPE II DYSLIPIDEMIA

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Background. Plasma high-density lipoprotein cholesterol (HDL-c) levels are inversely related with the risk of cardiovascular disease. Pharmaceutical modalities to raise HDL-c are scarce and show poor efficacy. However, inhibition of cholesteryl ester transfer protein (CETP), a plasma factor which mediates the transfer of lipids between lipoproteins, has been shown to effectively raise HDL-c. In clinical practice, CETP inhibitors are likely to be used in combination with evidence-based LDL-c lowering drugs. This study examines the safety and efficacy of the CETP inhibitor JTT-705 when combined with pravastatin. **Methods**. In a randomized, double-blind, placebo-controlled trial, 155 individuals with LDL-c>160 mg/dl using 40mg pravastatin, were treated with placebo, 300mg or 600mg JTT-705. **Findings**. After four weeks, 600mg JTT-705 led to a 30% decrease from baseline in CETP activity, a 28% increase from baseline in HDL-c ($p<0.001$ for both parameters compared to placebo), and a 5% decrease from baseline in LDL-c ($p<0.03$). In addition, decreases of 11% and 23% in LDL-c/HDL-c ratios were noted in the low and high dose groups, respectively. Increases in HDL2 (48%) and HDL3 (19%) in the high dose group, were accompanied by elevated apolipoprotein (apo) AI levels. In contrast, total cholesterol, triglyceride, apoB, and apoE levels were similar across the treatment groups. The combination therapy of JTT-705 with pravastatin did not raise notable safety concerns or significant adverse effects. **Interpretation**. Combination therapy of the CETP inhibitor JTT-705 with pravastatin effectively raises HDL-c levels and is safe and well-tolerated up to 4 weeks of administration by 101 dyslipidemic patients. Further studies will learn whether long-term treatment is safe and will yield the anticipated anti-atherogenic effects.

DISCOVERY OF A NOVEL MOLECULAR TARGET FOR POTENT HYPOCHOLESTEROLEMIC DRUGS: ROLE OF HISTONE DEACETYLASES

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High levels of plasma cholesterol increase the incidence of atherosclerosis. Cholesterol is disposed mainly via its conversion to bile acids. Here we describe the discovery of a novel target for the design of new hypocholesterolemic drugs. We dissected the transcriptional mechanisms of regulation of the gene (*CYP7A1*) encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid synthesis. Bile acids, the most important physiological regulators of cholesterol 7α-hydroxylase, repress *CYP7A1* gene transcription by recruiting specific histone deacetylases (HDACs) on this promoter. *In vivo* studies in the low-density lipoprotein receptor deficient (*Lld-r*$^{-/-}$) mouse, an animal model of hypercholesterolemia, shows that the HDAC inhibitor Valproic Acid (VPA) strongly increases the mRNA levels of *Cyp7a1*. The upregulation of *Cyp7a1* reduces total plasma cholesterol (204 ± 35 mg/dl in control mice vs. 29 ± 7 mg/dl in VPA treated mice, n=7, P<0.00001), reflecting increased cholesterol catabolism to bile acids. FPLC analysis of lipoprotein fractions shows that VPA decreases LDL in *Ldl-r*$^{-/-}$ mice. The specific effect of VPA on *Cyp7a1* transcription was assessed by measuring the mRNA levels of other genes involved in lipid metabolism, such as HMG-CoA reductase and apolipoproteins A-I, B and C-III. VPA also decreases plasma glucose and triglyceride levels. Taken together, our results demonstrate that inhibition of HDAC activity is a novel approach to lower plasma cholesterol by increasing bile acid synthesis and opens new avenues for effective treatment of hypercholesterolemia. [*Supported by grants from EC, 5FP QLG1-CT-2001-01513 and MIUR COFIN-PRIN 2002062991. ABVC is a fellow supported by a Marie Curie training program from the EC. This discovery was patented by the University of Milano*]

BERBERINE IS A PROMISING NOVEL CHOLESTEROL-LOWERING DRUG WORKING THROUGH A UNIQUE MECHANISM DISTINCT FROM STATINS

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Berberine (BBR), a compound isolated from a Chinese herb, has been widely used as a non-prescription drug to treat diarrhea in China for many years. Here, we identify BBR as an effective cholesterol-lowering drug. Oral administration of BBR in 32 randomly chosen hypercholesterolemic patients for 3 months reduced the serum cholesterol by 29%, triglyceride by 35%, and LDL-cholesterol by 25% without affecting serum HDL-cholesterol levels. BBR was well tolerated by all patients and no side effects were observed during the treating period. By conducting studies in human hepatoma cells we show that BBR strongly increases LDL receptor (LDLR) mRNA and protein expression. BBR upregulates LDLR expression independent of intracellular cholesterol levels and it has no effect on the processing of sterol regulatory element binding proteins. However, BBR directly stimulates the ERK signaling pathway and ERK activation is a prerequisite for the upregulation of LDLR expression by this drug through a posttranscriptional mechanism that stabilizes the mRNA. Utilizing a heterologous system with luciferase as a reporter, we further identify the 5' proximal section of the LDLR mRNA 3' untranslated region responsible for the regulatory effect of BBR. To corroborate our clinical and in vitro findings in animal models, the activities of BBR were examined in hamsters. We show that administration of BBR in hyperlipidemic hamsters for 10 days reduced serum cholesterol and LDL-cholesterol by up to 40% and 42% with 2.6-fold increases in LDLR mRNA and protein in the livers. Collectively, these findings obtained from clinic, animal model, and mechanistic studies strongly suggest BBR is a promising novel hypolipidemic drug that reduces serum LDL-cholesterol through a unique regulatory pathway distinct from statins, the current therapeutics for hypercholesterolemia.

PROTECTIVE ROLE OF SYNTHETIC HDL CONTAINING APOLIPOPROTEIN A-I$_{MILANO}$ FROM ISCHEMIA REPERFUSION INJURY: *EX VIVO* AND *IN VIVO* MODELS

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Myocardial injury is a potential consequence of coronary artery revascularization. We hypothesized that a synthetic HDL preparation containing apolipoprotein A-I$_{Milano}$ (ETC-216) may protect the heart from reperfusion injury. The *ex vivo* model consisted of rabbit hearts perfused by the Langendorff method. Hearts were equilibrated with buffer (10min), pretreated with ETC-216 (0.45mg/ml) or vehicle (10min), subjected to global ischemia (30min), and reperfused for 60min. ETC-216 prevented left ventricular end-diastolic pressure (p<0.05) and coronary artery perfusion pressure (p<0.001) compared to vehicle. ETC-216 reduced the release of creatine kinase (p<0.001). Electron microscopy revealed ETC-216 prevented mitochondrial granulation and sarcomere contraction band formation. ETC-216 (100, 10, 3mg/kg) was assessed for its cardioprotective effects in an *in vivo* model of left anterior descending artery (LAD) occlusion and reperfusion. ETC-216 or vehicle was infused intravenously for 60min beginning 15min before 30min of LAD occlusion and extending 15min into the 4h reperfusion period. Infarct area (IA) as percent of the left ventricle (LV) was significantly smaller in animals treated with ETC-216 at 100 (p<0.05), 10 (p<0.0001) or 3mg/kg (p<0.01) compared to vehicle. In a second protocol, ETC-216 (10mg/kg) or vehicle was infused intravenously for 60min beginning 5min before the end of 30min of LAD occlusion and extending 55min into the 4h reperfusion period. IA as percent of LV was significantly smaller in animals treated with ETC-216 (p<0.0005). Electron microscopy confirmed the *ex vivo* results. The findings suggest ETC-216 reduces reperfusion injury.

INHIBITORS OF ASBT INHIBIT TAUROCHOLATE UPTAKE IN HAMSTER AND HUMAN ILEAL PIECES

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Interruption of the enterohepatic circulation of bile salts lowers plasma cholesterol levels. The apical sodium-dependent bile acid transporter (ASBT), otherwise known as ileal bile acid transporter (IBAT), is a novel target for this purpose, and its inhibitors are expected to improve the demerits of resins, namely, the low compliance due to the high dosage and gastrointestinal side effects. In the course of our ASBT inhibitor research program, a series of 4-oxo-1-phenyl-1,4-dihydroquinolines showed strong ASBT inhibitory activities and were structurally different from the hitherto known ASBT inhibitors. Among them, R-146224 was a potent and specific inhibitor of ASBT (IC_{50} = 23 nM) in cells transfected with the human ASBT gene. It was approximately 1000-fold more potent than observed at inhibiting NTCP, a bile acid transporter in the liver. R-146224 also inhibited the uptake of [^{3}H]- taurocholate (TCA) in excised hamster ileal rings with the IC_{50}-value of 0.73 μM. Furthermore, a 14-day oral treatment with R-146224 (0.3-100 mg/kg) in chow-fed male Syrian golden hamsters caused significant reduction of serum non-HDL cholesterol by 12-37 % in a dose dependent manner.
To elucidate their effects in human, we tested the inhibitory potency of R-146119, R-146224 and R-150761 on ASBT in vitro by measuring the uptake of [^{3}H]- TCA in 5 x 5 mm pieces of freshly obtained human ileal segments. The materials were obtained from the resection of e.g. colon tumor tissue, conducted according to the rules of the local Medical Ethics Committee. TCA-uptake measurements in human ileal pieces are a slight modification of that in hamster ileal rings as recently described (Kurata et al., Bioorg.Med.Chem.Lett. 2004;14:1183-6). Incubations with the test compounds were performed in triplicate at 5 different concentrations. Each compound was tested in ileal segments from at least three different individuals. Average IC_{50}-values calculated after curve-fitting of the individual dose-response curves for R-146119, R-146224 and R-150761 were 1.41 μM, 0.46 μM and 0.42 μM, respectively, which were similar to those observed in hamster ileal samples.. Since a good correlation between serum cholesterol reduction and ASBT inhibitory activity in the ileal pieces was observed in the animal model, these compounds are also expected to show sufficient serum cholesterol reduction in human.

HMR1453 AS A NOVEL ILEAL BILE ACID TRANSPORTER (IBAT) INHIBITOR: PHARMACOLOGY IN MODELS OF ILEUM PERFUSION IN SITU AND FECAL BILE ACID EXCRETION AND HYPERCHOLESTEROLEMIC GUINEA PIG IN VIVO.

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Inhibition of intestinal bile acid reabsorption is an approach for lowering LDL-cholesterol. HMR1453, which itself exhibits a low absorption rate from the GI tract, represents a novel type of an inhibitor at the ileal apical sodium dependent bile acid transporter. (1) In the anaesthetized and bile duct cannulated male Wistar rat and male Syrian hamster HMR1453 inhibited in the in situ perfusion of the ileum with 3 mM taurocholic acid (TCA) containing buffer labeled with about 1000 dpm/µl ^{3}H-TCA, the appearance of ^{3}H-TCA in the bile in a concentration dependent manner. EC_{50} which inhibited the maximal ileal transport of ^{3}H-TCA by 50% versus a preceding control phase, was calculated at 0.78 (CI: 0.49 -1.37) µM/L in the rat and 7.9 (CI: 3.19-11.69) µM in the hamster. Washout after 10 µM HMR1453 with perfusion buffer only lead to complete reversal of inhibition. (2) In vivo, treatment of rats with HMR1453 at the dose range 0.2 to 25 mg/kg b.w./day p.o. increased fecal excretion of ^{14}C-taurocholic acid (^{14}C-TCA) in a dose related manner and was consistent in a number of experimental sets. A dose response curve constructed from N=2-5 of experimental dose groups with n=4-5 animals per dose group resulted in an ED_{200} (effective dose which increased fecal ^{14}C-TCA excretion up to 200% (control =100%)) of about 1.28 (CI: 0.54-2.92) mg/kg/day p.o. (3) In a model of hypercholesterolemia in the Dunkin Hartley guinea pig simultaneous feeding with HMR1453 in the feed at 0.01 –0.3 % over two weeks attenuated the development of hypercholesterolemia.
In conclusion, HMR 1453 interferes in a reversible manner with uptake of bile acids in the ileum and offers a therapeutic option for treatment of hypercholesterolemia via increased fecal excretion of bile acids.

LONG-TERM TREATMENT WITH THE NO-DONOR MOLSIDOMINE REDUCES CIRCULATING ICAM-1 LEVELS IN PATIENTS WITH STABLE ANGINA

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Recent clinical evidence has indicated that the severity of atherosclerosis is correlated with the level of soluble ICAM-1 (sICAM-1). Nitric oxide (NO) donors are used to treat patients with stable angina pectoris and the aim of this study was to investigate the short and long-term effect of molsidomine on the level of this circulating biochemical marker of endothelial function. We included 172 patients and examined the effect of the NO donor treatment on angina related parameters and on sICAM-1 levels after a 4 week and a one-year treatment period. After 4 weeks, angina attacks and sublingual (s.l.) isosorbide dinitrate tablet (ISDN) consumption frequency was significantly ($p<0.0001$) reduced without altering sICAM-1 levels when compared to the baseline values. The anti-anginal effect of molsidomine 16 mg o.a.d. was sustained (s.l. ISDN consumption) or improved (angina attacks frequency $p<0.002$) during the following year and a significant decrease in sICAM-1 levels ($p<0.0001$) was observed. When the sICAM-1 changes during the one-year treatment period were distributed in 4 categories (quartiles of the distribution), it was demonstrated that the decrease in s.l. ISDN consumption between the start and the end, was most pronounced in the group with the largest sICAM-1 decrease (4th quartile of distribution; $p=0.038$). In conclusion, the reduction in the proinflammatory marker sICAM-1 after one-year daily treatment with molsidomine may indicate that this NO donor besides its anti-anginal function, promotes a less activated state of the endothelium and thereby may modulate the progression of atherosclerosis in patients with stable angina.

TWO-YEAR EFFICACY AND SAFETY OF STATIN THERAPY IN HYPERCHOLESTEROLEMIC CHILDREN

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Children with familial hypercholesterolemia (FH) have increased carotid intima-media thickness (IMT). Long-term efficacy and safety of cholesterol-lowering medication has not been investigated in children.
Methods Therefore, we evaluated in a randomized, double-blind, controlled, two-year trial the efficacy and safety of pravastatin (20-40mg) in 214 children (8-18yr) with FH (106 received pravastatin, 108 placebo). The primary efficacy outcome was defined as the change from baseline in mean carotid IMT between the two groups over two years, whereas the principal safety outcome was measurement of growth, maturation and hormone levels over two years as well as changes of levels of muscle and liver enzymes.
Results Compared to baseline, carotid IMT showed a trend towards regression on pravastatin (Δ IMT: -0.010 ± 0.048 mm: p=0.049), whereas a trend towards progression was observed in the placebo group (Δ IMT: +0.005 ± 0.044 mm: p=0.280). The predefined primary efficacy outcome, namely the change of IMT between the two groups (0.014 ± 0.046 mm) did differ significantly (p=0.019). Also, pravastatin significantly reduced mean LDL-C levels compared to placebo (-24.1% versus +0.3%, respectively: p<0.0001). No differences were observed for growth, muscle or liver enzymes, endocrine function parameters, Tanner staging scores, and onset of menses or in testicular volume between the two groups.
Conclusions Two years of pravastatin induced a significant regression of carotid atherosclerosis in FH children with no adverse effects on growth, sexual maturation, hormone levels, liver or muscle tissue.

CETP IN RELATION TO LIPIDS, LIPOPROTEINS, FENOFIBRATE TREATMENT, AND CORONARY ANGIOGRAPHY IN PATIENTS WITH TYPE 2 DIABETES

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Cholesteryl ester transfer protein (CETP) transfers lipids between lipoproteins in human plasma. We here addressed the relationship between CETP, dyslipidemia, fenofibrate therapy, and coronary atherosclerosis in the Diabetes Atherosclerosis Intervention Study cohort. 341 patients of the DAIS population were stratified according to quartiles of baseline CETP concentration whereby the middle two quartiles were pooled. At baseline, increasing CETP levels were associated with a detrimental lipid profile characterized by increased triglycerides (p=0.018), VLDL-triglycerides (p=0.003) and smaller LDL size (p=0.001). Fenofibrate or placebo did not significantly affect HDL-c in each of the CETP quartiles. In the active arm, fenofibrate significantly reduced baseline total cholesterol levels in patients with lowest and median CETP concentration (-15% and -11%, respectively; p<0.0001 for both) but not in those starting with highest CETP at baseline (-5%, p=0.46). Similar results were found for LDL-c: -15% (p=0.0003), -6% (p=0.015) and 0.3% (p=0.65) for the low, median and high CETP groups respectively. In contrast, reductions of triglyceride-rich lipoproteins in the active arm of the study were similar across all quartiles. After 3 years of fenofibrate therapy, baseline CETP concentration was positively associated with loss of mean segment diameter (p=0.011), loss of minimum luminal diameter (p=0.037), and percentage diameter stenosis (p=0.051). Fenofibrate did not affect CETP concentration. In conclusion, CETP concentration is associated with a detrimental lipoprotein and increased progression of coronary atherosclerosis patients with type II diabetes treated with fenofibrate. This effect could, in part, be explained by an increased cholesterol-lowering effect of fenofibrate in patients with lower CETP concentration compared to those with higher CETP levels.

EFFECT OF RIMONABANT ON WEIGHT REDUCTION AND WEIGHT MAINTENANCE:RIO-EUROPE (RIO-EU) TRIAL

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Background: Rimonabant is the first selective cannabinoid type 1 (CB_1) blocker developed to manage cardio-vascular risk factors such as obesity and smoking. Preclinical studies demonstrated the role of the endocannabinoid system (ECS), via the CB_1 receptor, in the central and peripheral regulation of energy balance, as well as in the control of nicotine dependence. Furthermore, phase 2 studies established the efficacy of rimonabant in obesity and smoking cessation. Two studies RIO-EUROPE (RIO-EU) and RIO-NORTH-AMERICA (RIO-NA) have been designed to assess, over 2 years, the effect of rimonabant on weight reduction, weight maintenance and prevention of weight regain (RIO-NA) in overweight/obese patients with or without comorbidities. Both trials are part of a large program of 4 phase III trials conducted in over 6,000 overweight/obese subjects for up to 2 years. A recently completed trial in obese or overweight subjects with untreated dyslipidemia (RIO-Lipids) showed marked weight loss with positive effects on lipid and glycemic profiles together with a favourable safety profile suggesting that rimonabant may become an important tool in the management of cardiovascular risk factors. A fourth study (RIO-Diabetes) is conducted in obese patients with type 2 diabetes treated with monotherapy (biguanide or sulfonylurea).

Methods: RIO-EU is a multicenter, international, randomized, double-blind, placebo-controlled, parallel-group, fixed dose study to assess the effect of rimonabant on weight reduction and weight maintenance. Randomization was done after a 4-week single-blind placebo run-in period in subjects with BMI≥ 30 kg/m² or BMI>27 kg/m² with comorbidities. Patients with type 2 diabetes were excluded. Subjects were randomized for 2 yrs to receive either rimonabant 5 mg (R5) (n=604), rimonabant 20 mg (R20) (n=599) or placebo (P) (n=305), using a randomization ratio of 2:2:1. A mild hypocaloric diet was prescribed throughout the study. Primary efficacy endpoint is weight loss and weight maintenance at 1 yr. Secondary efficacy outcomes include lipid concentration, OGTT, fasting glucose /insulin homeostasis. Safety was evaluated throughout the study.

Baseline data: 1,507 patients (1198 women and 309 men, mean age 45.0 years, mean BMI 36.6 kg/m², mean weight 102.7 kg, mean waist circumference 109.9 cm) were randomized and treated. Forty one percent of them had hypertension and 61% had dyslipidemia. Forty one percent of the population met the clinical trial criteria of the metabolic syndrome (NCEP, ATPIII definition). Analyses are in process.

TYPE 2 DIABETES: A GLUCOSE OR LIPID DISEASE?

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The number of diabetic patients is increasing in an extraordinary manner. While there is no evidence that increased sugar consumption is associated with this phenomenal outburst of the disease, it is the progressive increase in human body weight that appears to drive diabetes pandemia. Obesity dramatically affects insulin sensitivity with respect to both glucose and FFA metabolism. The increase in the circulating plasma levels of glucose and FFA have deleterious effects (gluco- and lipotoxicity) at the level of many organs and tissues. Even in normal individuals experimental elevation of plasma glucose or FFA are sufficient to cause significant reduction in insulin sensitivity. The cellular mechanisms responsible for impaired insulin sensitivity involves activation of the hexosamine pathway, and down-regulation of the glucose transport system. Hyperglycemia and high FFA levels also contribute to the vascular complications of Type 2 diabetic patients. Endothelium-dependent vasodilation is altered in response to both acute and chronic exposure to glucose and FFA, while studies carried in animals demonstrate the existence of a gluco- and lipotoxic heart. Free-fatty acid, however, may have quite a critical role in the development of diabetes because they also exert deleterious effects on the beta-cell function. In an effort to better understand the phenomenon of lipotoxicity in human beta-cells, we have cultured human pancreatic islets with 1.0 or 2.0 mmol/l free fatty acid and showed a reduction in insulin content as well as glucose-stimulated insulin release. These changes were accompanied by a significant reduction of glucose utilization and oxidation. Apoptosis was increased mainly due to caspase activation. These findings are very much alike those observed in islets isolated from Type 2 diabetic patients. More recently, we have reported that lipotoxicity may also affect beta-cell function through suppression of PPAR-gamma expression. Thus, if islet are incubated in the presence of natural (PJ2) or synthetic (glitazones) most of the deleterious effects of FFA are prevented due to the activation of PI-3-k activity.

In summary, both epidemiologic, physiologic, cellular, and molecular studies point out the important role of lipotoxicity in determining the risk for Type 2 diabetes and associated cardiovascular disease. This should not, however, underscore the acceleration of this process induced by hyperglycemia. Both in vivo and in vitro studies indeed confirm that glucose elevation plays an important role in causing the characteristic progressive metabolic deterioration in the natural history of Type 2 diabetes.

LIPOPROTEIN/FREE FATTY ACID PARTITIONING IN SKELETAL MUSCLE AND INSULIN RESISTANCE

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Except for white adipose tissue where triglyceride (TG) storage is the predominant fate, fatty acid uptake into tissues is an important contributor to the oxidative fuel needs of the tissue. The two sources of uptake from plasma include albumin bound free fatty acids (FFA) and lipoprotein TG fatty acids through the action of lipoprotein lipase (LPL). An alternative source of fatty acids is the TG storage pool within myocytes. When the breakdown of intramyocellar TG is modified in the absence of hormone sensitive lipase, neutral lipid accumulation and reductions in insulin action in skeletal muscle are seen. Of interest, a similar phenotype exists when muscle-specific overexpression of the fatty acid transporter CD36 or LPL is created. The most likely mechanism for this insulin resistance is a defect in insulin-mediated glucose uptake by skeletal muscle. Candidate cellular mediators of this effect include fatty acid induced increases in DG and/or fatty acyl-CoAs with downstream activation of protein kinase C-θ and subsequent increases in serine vs tyrosine phosphorylation. Using DNA array approaches, we have recently found that mice transgenic for muscle-specific overexpression of LPL have insulin resistance and increases in the SNARE accessory protein Munc18c gene expression (Schlaepfer IR et al. J. Lipid Res. 2003;44:1174-81). Similar increases were seen in high fat fed and diabetic mice, and in C2C12 myoblasts stably transfected with LPL. These cells have been recently utilized to determine if LPL-derived fatty acids have similar metabolic fates as albumin bound FFA. Of interest, fatty acids resulting from the hydrolysis of TG-rich lipoproteins by LPL are preferentially directed to TG accumulation whereas a greater % of FFA appears to be partitioned to oxidative pools. Separate pathways of lipoprotein TG fatty acids vs FFA have been shown in the heart by the Goldberg group (Augustus AS et al, Am J. Phys Endo Metab 2003;284:E331-9). Overall, this would suggest a rationale for why skeletal muscle LPL is down-regulated in insulin resistant states but up-regulated after TG depletion following sustained exercise.

INCREASED FATTY ACID AND GLUCOSE METABOLISM IN CULTURED HUMAN MYOTUBES AFTER TREATMENT WITH THE LIVER X RECEPTOR (LXR) AGONIST T0901317

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Little is known about the role of the LXRs in skeletal muscle. We investigated the effects of T0901317 on lipid and glucose metabolism in myotubes from obese type 2 diabetics (T2D) and lean healthy matched controls. Differentiated myotubes were exposed to T0901317 (0.1-1 μM) for 4 days, and then incubated for 4 h with [^{14}C]palmitic acid (PA), [^{14}C]glucose or [^{3}H]deoxyglucose. T0901317 increased PA uptake by 50 % (45 nmol/mg protein) in control myotubes. Interestingly, the increment was 31 % higher (59 nmol/mg protein) in T2D myotubes. PA incorporation to DAG, TAG and PL was 2-fold increased in control cells, whereas T2D myotubes showed an additional 70-80 % increase. T0901317 increased PA oxidation to CO_2 by 22 %. This effect was absent in T2D cells. Insulin-stimulated glucose transport was elevated by 30 % and glucose oxidation was increased by 65 % after T0901317. No differences were observed between T2D and control myotubes, except that glycogen synthesis was decreased by 30 % in T2D cells. The mRNA expression was up-regulated after T0901317 for CD36/FAT (2-3-fold), ACS-2 (3-fold), GLUT4 (6-fold), LXRα (8-fold) and PPARγ (3-fold). LXRβ was 50 % higher in control myotubes. Our data show that chronic T0901317 increases lipid accumulation in human myotubes. The effect is stronger in T2D than control myotubes, and this might involve an absent increase in lipid oxidation. Moreover, T0901317-incubation had a positive effect on insulin-stimulated glucose metabolism for both groups. These findings might imply that dysregulation of the LXR pathway could be attributing to the increased intramyocellular lipid level found in T2D subjects.

EFFECT OF COLIPASE INHIBITOR ON FEEDING AND CIRCULATING LIPIDS IN RATS

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Colipase inhibitor, a novel potent specific non-covalent inhibitor of lipase, has been shown to inhibit lipase activity and lipolysis in *in vitro* studies. To investigate whether colipase inhibitor has lipid lowering properties *in vivo*, in the first experiment, a single dose of colipase inhibitor with or without intralipids (200mg) was given orally through a gastric gavage to female Sprague-Dawley rats that had been fasted overnight. Administration of colipase inhibitor resulted in a highly significant reduction in serum triglycerides and fatty acids levels during the test period of 180 minutes, but had no effect on serum cholesterol levels. A second experiment was subsequently undertaken to determine the effect of administration of colipase inhibitor on food intake and body weight. Colipase inhibitor was orally administered for 5 days at three doses (25, 37.5 and 50 µl) to rats fed either a high-fat diet or a low-fat diet. Serum triglycerides and fatty acids level were markedly reduced in high-fat feeding rats, but serum cholesterol levels were similar in colipase inhibitor-treated rats compared with control rats. The reduction of food intake was accompanied with the decreased body weight in high-fat feeding rats. Interestingly, colipase inhibitor failed to decrease food intake and body weight in rats fed with a low-fat diet. To explore the mechanism by which colipase inhibitor inhibited food intake and decreased circulating lipids, we have measured peptides that have been demonstrated to involve appetite regulation, such as PYY_{3-36}, leptin and ghrelin. Serum leptin levels were significantly decreased and serum ghrelin levels increased in colipase inhibitor-treated rats when compared to the control rats. By contrast, there was no significant difference of serum PYY_{3-36} levels between colipase inhibitor and control group (0.85±0.26 ng/ml vs.1.1±0.24ng/ml). Furthermore, we found that the protein expression of pancreatic lipase was reduced in colipase inhibitor-treated rats. The mechanism behind the suppression of food intake and circulating lipids by colipase inhibitor is unknown yet. However, the potential use of this colipase inhibitor as a therapeutic tool against hyperlipidaemia and obesity is emerging by the effectiveness of reduction in body weight, food intake and blood lipids.

OVEREXPRESSION OF MITOCHONDRIAL GLYCEROL-3-PHOSPHATE ACYL TRANSFERASE (MTGPAT) INCREASES HEPATIC LIPID SYNTHESIS AND INHIBITS BETA OXIDATION

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Glycerol-3-phosphate acyl transferase (GPAT) catalyses the first committed step in glycerolipid biosynthesis. The mitochondrial isoform (mtGPAT) is mainly expressed in liver where it is highly regulated, indicating that mtGPAT may have a unique role in hepatic fatty acid metabolism. Since both mtGPAT and carnitine palmitoyl transferase-1 (CPT-1) are located on the outer mitochondrial membrane, we hypothesized that mtGPAT directs fatty acyl-CoA away from β-oxidation and towards glycerolipid synthesis. Adenoviral-mediated overexpression of murine mtGPAT in primary cultures of rat hepatocytes increased mtGPAT activity 2.7-fold with no compensatory effect on microsomal GPAT activity. MtGPAT overexpression resulted in a dramatic 80% reduction in fatty acid oxidation and a significant increase in hepatic diacylglycerol and phospholipid biosynthesis. Mice were infected with control adenovirus or mtGPAT expressing adenovirus and hepatic lipids analyzed. Results demonstrate that livers from mtGPAT infected mice were larger and enhanced with diglycerides and triglycerides. Hepatic mtGPAT activity was increased 4-fold as compared with control mice demonstrating that overexpression of mtGPAT increases hepatic lipid accumulation. Overall, these results support the concept that increased hepatic mtGPAT activity positively contributes to lipid disorders by reducing fatty acid oxidation and promoting *de novo* glycerolipid synthesis.

SELECTIVE INHIBITION OF HORMONE SENSITIVE LIPASE DECREASES PLASMA FREE FATTY ACIDS AND GLYCEROL

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Hormone sensitive Lipase (HSL) is the rate limiting enzyme of intracellular triglyceride hydrolysis and fatty acid release in adipocytes. It is regulated by signalling pathways triggered by adrenalin, noradrenalin and glucagon and counter-regulated by insulin. The strict control by hormones as well as by the sympathic and parasympathic nervous system outlines its pivotal role in lipid metabolism and energy homeostasis.
In obese type II diabetic patients HSL activity is dysregulated and results in an elevated flux of free fatty acids (FFA) from adipocytes to muscle, liver and ß-cells, where they can be oxidized to a certain degree. However, the still remaining high levels of FFA are discussed to impair peripheral insulin action and insulin release from the β-cells. Thus, the inhibition of HSL may offer a pharmacological approach to reduce FFA levels resulting in diminished peripheral insulin resistance and improved ß-cell function.
Consequently, we have developed small orally available molecules, which inhibit human and rat HSL with high potency (IC_{50} 1-10 nM) and selectivity (against other lipases, esterases and proteases). These compounds reduce basal and isoproterenol-induced lipolysis in rat and human adipocytes with IC_{50} values in the range of 0,1-1µM (release of glycerol and fatty acids). The antilipolytic activity of these inhibitors was demonstrated in rodents and dogs. Characterization of the mode of inhibition was performed using recombinant human HSL and subsequent proteomic investigation.
The pharmacodynamic and pharmacokinetic profile of these compounds may enable pharmacological trials designed to demonstrate HSL inhibition as a novel target for antidiabetic therapy.
References: Müller and Petry (eds), Lipases and Phospholipases in Drug Development 2004, Wiley-VCH, Weinheim, Germany

APOLIPOPROTEIN C3-DEFICIENCY RESULTS IN DIET-INDUCED OBESITY AND INSULIN RESISTANCE IN MICE

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Aim: Apolipoprotein C3 (apoC3) is a strong inhibitor of lipoprotein lipases (LPL), a key enzyme in fatty acid delivery to muscle and adipose tissue. Our aim was to study whether the absence of apoC3 accelerates the development of obesity and insulin resistance. Methods: apoc3-/- mice and wild type littermates were fed a high fat (46 energy %) diet for 20 weeks. Body weight and food intake were assessed weekly. Adipose tissue fatty acid uptake and body fat composition were analyzed at the end of the study. Insulin sensitivity was determined using hyperinsulinemic euglycemic clamps with 3H-glucose as a tracer. Results: After 20 weeks of high fat feeding apoc3-/- mice were more obese than wild type littermates (42.8 ± 3.2 vs. 35.2 ± 3.3 g, $p<0.05$). This increase in body weight was entirely explained by increased body lipid mass (16.2 ± 5.9 vs. 10.0 ± 1.8 g, $p<0.05$). No significant difference in adipocyte size was observed as compared to wild type littermates. In adipose tissue fatty acid uptake from plasma triglycerides (TG) was significantly higher in apoc3-/- mice, whereas uptake of albumin-bound fatty acids, used as a measure to determine LPL-independent uptake of fatty acids, did not differ from that of control mice. Interestingly, whole body insulin sensitivity was decreased by 43% in apoc3-/- mice compared to wild type littermates. Hepatic insulin sensitivity was decreased by 26% in apoc3-/- mice compared to control mice. Conclusion: Absence of apoC3, the natural LPL inhibitor, enhances fatty acid uptake from plasma TG in adipose tissue, which in turn leads to higher susceptibility to diet-induced obesity and insulin resistance. Therefore, apoC3 is a potential target for treatment of obesity and insulin resistance.

ENDOTHELIAL PHENOTYPES IN HEALTH AND DISEASE: GENOMIC APPROACHES

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The endothelial cells (EC) that line the cardiovascular system comprise a vital, multifunctional interface in health, and their dysfunction contributes to inflammation, hypertension, thrombosis, and atherosclerosis. EC dysfunction can be elicited by biochemical stimuli, such as proinflammatory cytokines, bacterial endotoxins, advanced glycation endproducts, or components of oxidized lipoproteins. In addition, biomechanical forces generated by the pulsatile flow of blood (wall shear stresses) can also directly influence EC phenotype. Our laboratory has applied genome-wide phenotypic profiling, via cDNA and oligonucleotide microarrays, to compare the patterns of gene expression induced by biochemical stimuli and biomechanical stimuli in human EC cells under well controlled experimental conditions. This approach has defined distinct and reproducible patterns of gene regulation that are associated with pathophysiologically relevant endothelial activation states, and has identified novel genes encoding cell surface receptors, ion transporters, signaling molecules and transcription factors that have selective endothelial expression (http://www.vessels.bwh.harvard.edu). The extension of these strategies to normal and diseased human and mouse blood vessels should help provide new insights into the mechanisms of cardiovascular disease, and hopefully reveal novel molecular targets for therapeutic interventions.

MOLECULAR GENETIC STUDIES ON THE ROLE OF PROTEASES IN CARDIOVASCULAR DISEASE

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Human atherosclerosis is a complex disease evolving through the interaction between many genes and a wide range of environmental and lifestyle factors. Migration and proliferation of cells along with extensive deposition and modification of the extracellular matrix (ECM) are hallmarks of the atherosclerotic process and of the complications of plaque rupture and aneurysm formation. The remodelling of the ECM is under tight control by matrix-degrading proteases and their inhibitors.

The family of matrix-degrading proteases includes serine proteases, matrix metalloproteinases (MMPs), and cysteine and aspartic proteases. Plasmin, which is the major serine protease, has a broad substrate specificity, and its generation is primarily controlled by plasminogen activator inhibitor-1 (PAI-1). MMPs are a family of zinc- and calcium-dependent endopeptidases that have the capacity to degrade all of the ECM proteins. The MMP system is regulated at several levels: (1) by transcriptional activation through cytokines and growth factors, (2) by activation of inactive zymogens, and (3) through inhibition of the active enzyme by tissue inhibitors of MMPs (TIMPs). Less is known about the lysosomal cathepsins that are synthesised as inactive precursors that require proteolytic activation and are inhibited by cystein protease inhibitors, cystatins.

The past several years have seen a range of candidate gene studies focusing on proteases and protease inhibitors and combining clinical or epidemiological studies of the discriminatory value of new polymorphisms in clinical cohorts with in-vitro work to demonstrate functional effects on either gene expression or the protein product. A special emphasis has been placed on the search for polymorphisms in regulatory (promoter) regions. Such DNA variation may substantially alter gene transcription and is a potential basis for clinically important gene-environment interactions. Functional polymorphisms associated with relevant clinical phenotypes have so far been detected in the promoter regions of MMP-2, MMP-3, MMP-7, MMP-9, MMP-12, PAI-1 and cystatin C.

Functional molecular genetics has proven a useful tool for demonstrating the significance of proteases and protease inhibitors in atherosclerosis and related clinical complications.

EZETIMIBE TREATMENT REGULATES EXPRESSION OF INTESTINAL GENES RELATED TO CHOLESTEROL SYNTHESIS AND LIPID METABOLISM

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Many genes involved in cholesterol synthesis and metabolism are under tight regulatory control by the intracellular pool of cholesterol. Ezetimibe, a cholesterol absorption inhibitor, acts to prevent the entry of cholesterol from the intestinal lumen into enterocytes. Microarray analysis was used to investigate the effect of treating mice with ezetimibe on the expression of intestinal genes. Mice (n=7-9) were fed normal chow or chow with 1% cholesterol/0.5% cholate for 7 days, with daily dosing of 10 mg/kg ezetimibe or vehicle. RNA from the proximal half of the small intestine was hybridized to Agilent arrays. Genes that differed in expression (>1.5-fold, $p<0.01$) were compared for normal chow vs. high cholesterol diet: 321 increased expression and 349 decreased expression with cholesterol feeding. The regulated gene signature of ezetimibe treatment in a high cholesterol diet setting showed 92 genes increased and 253 decreased in expression. Few genes changed expression levels by more than two-fold. Most genes in common between the high cholesterol diet and ezetimibe treatment signatures were anti-correlated. Among genes up regulated by ezetimibe treatment were ones involved in cholesterol synthesis: squalene epoxidase, FDP farnesyl transferase, sterol reductase, and mevalonate kinase. Among those down regulated by ezetimibe treatment were genes involved in cholesterol transport and lipid metabolism: ABCA1, carnitine palmitoyl transferase, cytoplasmic thioesterase 1, two acylCoA dehydrogenases and a fatty acid transport protein 2.

GENE EXPRESSION IN TWO RABBIT MODELS OF DYSLIPIDAEMIA

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In this study we compared the gene expression in various tissues from the St Thomas' Mixed Hyperlipidaemic Rabbit (SMHL a model of FCH), Watanabe Heritable Hyperlipidaemic Rabbit (WHHL a model of FH), and control New Zealand White rabbits (NZW). This was done using real time quantitative PCR of a gene panel consisting of genes selected to be relevant to atherogenesis and dyslipidaemia. 90 genes were selected covering metabolic and inflammatory aspects of disease. Many rabbit gene sequences are not available in the literature so data mining and extensive cloning was undertaken to allow the design of Sybr primers, followed by Taqman primers and probes. An array-style plate containing gene-specific primers was set up in which a single cDNA sample is analysed for the expression of a panel of genes. As the first test of this approach, RNA extracted from liver samples from the three rabbit strains was converted to cDNA and the expression levels of the selected genes was measured.
A Partial Least Squares Discriminant Analysis of the gene expression in the liver showed that the three rabbit strains were clearly distinguishable. Use of the Variable Performance in the Projection statistic allowed an estimate of the importance of individual genes. Some genes were influential in discriminating both dyslipidaemic models from NZW and others were characteristic of the individual strains. For example, on this basis, ABCA1, the macrophage scavenger receptor and CRP were all influential in discriminating between the two dyslipidaemic strains and the NZW control.

ATHEROSCLEROSIS BEFORE AND AFTER TRANSFORMATION OF FATTY STREAKS INTO INTEGRAL PLAQUES—IMPLICATIONS FOR DISEASE EXPANSION AND GENE EXPRESSION

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Background—We examined atherosclerosis development and related changes in morphology and gene expression in mice with human-like hypercholesterolemia and a genetic switch to turn off hepatic lipoprotein synthesis (*Ldlr*$^{-/-}$*Apob*$^{100/100}$*Mttp*$^{flox/flox}$ Mx1-*Cre*).

Methods and Results—Atherosclerosis commenced slowly, expanded rapidly from 30 to 40 weeks, and plateaued thereafter. The rapid expansion was preceded by transformation of fatty streaks into integral plaques and expression of genes with atherogenic properties (VCAM, MCP-1, CD68, CD36, and MMP-2). Between 15 and 30 weeks, disease expansion was modest (~1.6%), but gene expression levels increased several fold, and the strong co-regulation of their transcriptional activity seen in fatty streaks was lost in plaques. Acute lowering of plasma apoB100-containing lipoproteins reduced atherogenic gene expression in mice with fatty streaks but caused unexpected activation in mice with integral plaques.

Conclusions—Transformation of fatty streaks into plaques results in a phenotypic switch characterized by rapid lesion expansion, gene activation, loss of co-regulation, and paradoxical responses to cholesterol lowering. These findings may have implications for the timing of lipid-lowering regimens in humans.

RXR-GAMMA POLYMORPHISM IS ASSOCIATED WITH ATHEROGENIC LIPID PROFILES AND CORONARY ARTERY DISEASE

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FCHL is most common inheritable hyperlipidemia. Recently, RXR-gamma gene is associated with LPL activity and weight gain in mice. RXR-gamma is located in FCHL locus chromosome 1q21-q23 in human. [Methods] We screened all the coding regions of RXR-gamma gene in 60 FCHL and 120 other primary hyperlipidemia by PCR-DGGE. Clinical characteristics of identified mutation were evaluated in 298 general males (LDL-C 119 ± 30, HDL-C 48 ± 12, TG 137 ± 91 mg/dl, BMI 23.6 ± 3 kg/m^2; mean ± SD) and in 105 coronary angiography (CAG) performed patients (M/F = 52/53) (LDL-C 135 ± 51, HDL-C 48 ± 13, TG 142 ± 64, RLP-C 4.6 ± 3, HDL2-C 28 ± 8, HDL3-C 15 ± 3 mg/dl, BMI 23.9 ± 3 kg/m^2; coronary stenosis index;CSI 12.5 ± 10) using PCR-RFLP. Post-heparin lipoprotein activities (PHLA) were measured in CAG group. [Results] Novel polymorphism RXR-gamma G14S was identified in patients with hyperlipidemia. 1) In general males, 14S carriers had significantly higher BMI (24.8 ± 2 kg/m^2, p=0.049) and tended to have higher TG levels (200 ± 209 mg/dl). 2) In CAG group, 14S carriers had significantly higher CSI (21.4 ± 6, p=0.05), higher RLP-C (8.0 ± 4 mg/dl, p=0.006), lower HDL-C (37 ± 9 mg/dl, p= 0.03), lower HDL2-C (21 ± 5 mg/dl, p=0.03). In ultracentrifugation analysis, 14S carriers had significantly higher TG and PL in VLDL. 14S carriers tended to have higher LDL-C, higher TG, higher BMI, lower LPL activity, and younger age at CAG. 3) 14S carriers were significantly frequent in FCHL (15%) than in general males (5%), other hyperlipidemia (4%), and CAG group (5%) (p=0.03). [Conclusion] RXR-gamma is an important genetic candidate of dyslipidemia including FCHL.

SELDI-TOF MS: A POTENTIAL HIGH-THROUGHPUT TOOL FOR HIGH DENSITY LIPOPROTEIN PROTEIN ANALYSIS

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Although in the last decade substantial progress in the etiology, prevention and treatment of vascular diseases has been made it remains the major cause of death in our modern western society. High Density Lipoprotein (HDL), one of the main plasma lipoproteins, serves as a docking station for a variety of factors involved in inflammation, coagulation, protective properties against lipid-oxidation and lipid metabolism. Yet, the link between HDL and exact nature of these mechanisms in relation to the development of atherothrombotic diseases are still not fully understood. Here we propose SELDI-TOF mass spectrometry as a high-throughput proteomic tool for the analysis of the protein composition of HDL associated proteins.

Antibodies against apo A-I and apo A-II were covalently bound to a PS20 protein chip and HDL of normolipemic subjects was immuno-captured. Sinapinic acid was used as a matrix to desorb bound molecules and facilitate mass spectrometry. The method was evaluated on reproducibility and robustness.

Within the used SELDI-chip batch the chip-to-chip variation was acceptable. Reproducible fingerprints could be produced up to 2 weeks after processing of the samples. Depending upon the wash stringency after HDL immunocapture, albumin contamination could be partially eliminated without loss of HDL "specific" components. However, serum and heparinized plasma gave rise to more abundant albumin in the protein fingerprint compared to EDTA or citrated plasma.

On-chip capture of HDL either from plasma or from gelfiltration pre-purified HDL resulted in comparable fingerprints, indicating specific capture of HDL particles. Depending on the used capture antibody (anti apo A-I or A-II) specific differences in the fingerprint were seen of the captured HDL (sub)populations. Similarities in HDL components were also observed from proteins which apparently are present in both HDL subpopulations. The most detailed fingerprint was observed up to 50 kDa, 15 to 20 separate proteins were detected in the 5-50 kDa molecular mass range. Between 50 and 160 kDa 5 to 10 more proteins were detected.

SELDI-TOF mass spectrometry of HDL may be a suitable candidate for high-throughput analysis of HDL protein composition. This approach may contribute to the investigation of the underlying mechanisms that lead to increased atherothrombotic risk and may serve to predict the atherothrombotic state of an individual.

ALCOHOL AND CARDIOVASCULAR DISEASE: RECENT EPIDEMIOLOGICAL DATA

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A large number of studies from many different countries have shown a J- shaped relation between alcohol intake and all-cause mortality. The descending leg of the curve is due to a decreased risk of cardiovascular disease among those who have a light to moderate alcohol intake. Persons with a high alcohol intake have a higher level of high density lipoprotein, which has been found to be a mediator of 40-60 % of the effect of alcohol on coronary heart disease. Recently it has been suggested that the J-shaped relation is influenced by different factors such as age, gender, genetic factors and drinking pattern. Hence, large American studies have shown that only the elderly and those already at risk of developing coronary heart disease are prevented from his by drinking alcohol. A few studies have suggested that some ADH-phenotypes are more susceptible to the beneficial and detrimental effects of alcohol than others. A large study from Australia has suggested that only those who have a steady - in contrast to a binge - intake of alcohol have benefits with regard to coronary heart disease mortality. The latter has very recently been supported by a large prospective study from Copenhagen. Correlational studies showed that mortality from coronary heart disease is lower in countries where wine is the predominant type of alcohol, than in countries where beer or spirits are the beverages mainly ingested. Recent prospective studies from United Kingdom, Sweden and Denmark have supported the above by showing that wine drinkers are at lower mortality than beer and spirits drinkers. Several of the components in wine which may have antioxidant proporties are also present in fruits and vegetables. Therefore, diet may play a role in the interpretation of the complex relation between alcoholic beverage type and coronary heart disease mortality. In the Danish Diet Cancer and Health Study, preference of wine was associated with a higher intake of fruit, fish, vegetables, salad and a higher frequency of use of olive oil for cooking compared with preference of beer or spirits in both men and women.

In conclusion, the complex association between alcohol and health seems even more complex after these suggestions of a number of effect modifiers such as age, sex, genetics and drinking pattern that wine drinkers are at a decreased risk of death from coronary heart disease than non-wine drinkers, suggest that substances present in wine are responsible for a beneficial effect on the outcome, in addition to that from a light intake of ethanol. However, several potential confounders remains to be excluded

MECHANISMS OF ANTI-ATHEROSCLEROTIC EFFECTS OF ALCOHOL: FOCUS ON ANTI-INFLAMMATORY ACTION

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An inverse association between moderate alcohol consumption and vascular risk has been shown in many epidemiological studies. All-cause mortality as a function of alcohol use has been depicted as a J-shaped curve, reflecting a lower risk of coronary heart disease (CHD) at moderate consumption and an increased risk of certain cancers and cirrhosis at higher amounts. After wine intake was suggested as a possible explanation for the lower than expected CHD mortality rates in France, many studies have dealt with the question of whether different alcoholic beverages are equivalent in their ability to protect against CHD or if a specific beverage might offer a greater protection. The beneficial effect of moderate drinking on atherosclerosis has usually been attributed to changes in the lipoprotein profile and the coagulation system. More recently, atherosclerosis has been considered a chronic low-grade inflammatory disease in which the adhesion of monocytes to endothelial cells, through the interaction of adhesion molecules expressed on both cells, plays a pivotal early event in pathogenesis. In vitro studies have shown that some compounds, such as flavonoids, found in alcoholic beverages can modulate monocytic cell adhesion to the endothelium. However, few data are available on the effects of various alcoholic beverages with different polyphenolic content on the early phases of atherosclerosis in humans. In a recent study TNF–induced adhesion of monocytes to endothelial cells was virtually abolished after red wine consumption but was only partially reduced after gin consumption. These findings raise the intriguing possibility that protective anti-inflammatory effects might be limited to specific alcoholic beverages.

NON-CARDIOVASCULAR EFFECTS OF MODERATE ALCOHOL CONSUMPTION

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The inverse association between alcohol and coronary heart disease is well documented. However, alcohol and alcohol metabolites also have many other important biological effects. Over the last decade there has been convincing evidence to suggest that alcohol in moderation may be beneficial to insulin sensitivity, body weight and ultimately may lower risk of type 2 diabetes. Benefits of moderate alcohol consumption have also been documented for gallstone disease, hypertension, benign prostatic hypertrophy, and erectile dysfunction. There are also equally convincing data that alcohol consumption, even at moderate levels, is associated with excess risk of cancer. For example, there are now over 10 well-conducted prospective studies which have found that excessive alcohol consumption (> 2 drinks/day) among women is associated with a higher risk of breast cancer. Even at lower levels of consumption, risk may be elevated. Interestingly, this may be mediated through the direct effects of alcohol on hormones or through the anti-folate effects of alcohol and its metabolite acetaldehyde. In several prospective studies, results suggest that women with sufficient dietary folate do not have an elevated risk of breast cancer associated with alcohol consumption. These same alcohol folate interactions have been reported for colon cancer. When summarized over all health effects, alcohol in moderation lowers risk of all-cause mortality by 20%. Although most of the benefit arises from cardiovascular disease, the other important biological effects of alcohol should not be ignored.

NON-ALCOHOLIC COMPOUNDS OF ALCOHOLIC BEVERAGES: BIOLOGICAL AND PREVENTIVE EFFECTS

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The hypothesis of a lower mortality from coronary heart disease (CHD) in wine (namely red wine) drinkers, as compared with subjects who consume beer, spirits, or no alcoholic beverages, is corroborated by several findings that indicate how wine phenolic constituents are endowed with potent "pharmacological" properties. Such activities include a strong antioxidant capacity (with the potential to inhibit LDL oxidation), inhibition of platelet aggregation, amelioration of vascular reactivity, reduction of lipid hydroperoxides in the stomach, and others. Indeed, red wine contains remarkable amounts (1-2 gr/Lt) of phenolic molecules, many of which yet to be fully identified. It must be noted that the vast majority of evidence in support of a healthful role of wine phenolics comes from in vitro studies. Conversely, the few animal or human studies performed thus far do not convincingly demonstrate any biological effect of wine consumption. This might in part due to the low absolute concentration of bioactive molecules, e.g. trans-resveratrol, catechins etc in red wine, to their poor bioavailability (at least of the ones identified in human plasma/urine), to the confounding effects of ethanol per se, or to the current lack of appropriate methodology to evaluate biological activities of individual components. In turn, even though biochemical evidence in support of a "wine theory" is strong, information on the human activities of wine minor constituents is currently too scant to draw scientifically sound conclusions.

ROLE OF STATINS IN ATHEROSCLEROTIC PLAQUE STABILIZATION: BEYOND CHOLESTEROL.

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Statins are widely used in the treatment of hypercholesterolemia, but besides lowering cholesterol levels, they effectively reduce cardiovascular events and mortality in humans. Angiographic studies reported that clinical benefits of statins are greater than those expected on the basis of the modest change in arterial stenosis severity. A potential mechanism of these effects is referred to as "plaque stabilization": reduction of cholesterol levels might modify the propensity for plaque rupture and thrombosis by changing plaque composition. We previously reported that the inducible isoforms of cyclooxygenase (COX-2) and PGE synthase (mPGES-1) are up-regulated in vulnerable plaques of symptomatic patients, and contribute to lesion instability through release of PGE_2–dependent metalloproteinases (MMPs), proteolytic enzymes capable of degrading plaque constituents. In a recent study, we analyzed plaques from 70 symptomatic patients randomized to simvastatin (40 mg/d) or placebo for 4 months. We found that plaques from simvastatin group had fewer inflammatory cells, lower levels of COX-2/mPGES-1, MMPs, lipids and oxLDL and higher collagen content as compared to plaques from control group. This study demonstrated that simvastatin-based therapy may promote changes in plaque composition toward a more stable phenotype, by reducing MMP release.
As diabetes is a major risk factor of atherosclerosis, we evaluated inflammatory profile of symptomatic plaques from diabetic patients. We observed higher levels of COX-2/mPGES-1 and increased MMP activity in plaques from diabetic patients. Moreover, we found that COX-2/mPGES-1 overexpression can be modulated by the expression of RAGE (receptor for advanced glycation end products), that is known to play a key role in the accelerated progression of atherosclerosis in diabetes. Thus, we reported that RAGE overexpression in atherosclerotic diabetic plaques may promote plaque instability by inducing PGE_2 biosynthesis, leading in turn to MMP release. On the basis of these data, we analyzed expression of RAGE and RAGE-dependent plaque-destabilizing genes in plaques from diabetic patients after simvastatin or placebo therapy. Our results suggested that simvastatin stabilizes diabetic plaques at least in part through down-regulation of RAGE expression, that leads in turn to inhibition of PGE_2-dependent MMP biosynthesis.

ASSESSMENT OF REACHING GOAL IN PATIENTS WITH COMBINED HYPERLIPIDEMIA: LDLC, NONHDLC, APO B OR RATIOS

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The 2001 NCEP-ATPIII guidelines introduced for the first time the concept of nonHDLC and set treatment goals for this parameter. The selection of nonHDLC, especially in those subjects with combined hyperlipidemia [HLP] (elevated cholesterol and triglyceride between 200-500 mg/dL) was proposed as a surrogate for apolipoprotein B (ApoB), which both epidemiological and clinical trial data indicate is the best predictor of future coronary events. However there is little data to compare the efficacy of lipid-lowering therapies on achieving ApoB versus nonHDLC targets.

We studied a cohort of more than 80,000 lipid measurements including more than 25,000 from subjects with combined HLP, on diet or a variety of lipid lowering agents, to determine the relationship between ApoB, nonHDLC and LDLC target achievement. NCEP-ATP III targets of <130 and <100 mg/dL for high risk, and 130-160 and 100-130 mg/dL for moderate risk groups for nonHDLC and LDLC respectively were compared to population equivalent values for ApoB of <90 and 90-105 mg/dL for these risk catagories. Plasma lipids and ApoB were measured in a CDC part III standardized laboratory. LDLC was measured by preparative ultracentrifugation (BQLDLC) and calculated by Friedewald formula (LDLCcalc).

A substantially lower percentage of patients achieved the ApoB targets than reach the nonHDLC or LDLC targets. More than twice as many patients achieved the LDLC than nonHDLC targets in the high risk category, while of those who reached the high risk nonHDLC target, 62% do not reach the ApoB target. Of the minority who reach the < 90 mg/dL apoB target, all have a BQLDLC <100 mg/dL, but only 82% reach the nonHDLC target of <130 mg/dL. We conclude that in patients with combined HLP and triglyerides 200-500 mg/dL, nonHDLC, LDLC and apoB are not equivalent targets and thus may not yield the same anticipated reduction in cardiovascular risk on therapy.

PLASMA REMNANTS LIPOPROTEINS AND INTIMA-MEDIA THICKNESS IN THE METABOLIC SYNDROME. THE PRESENCE AND PROGRESSION OF LESIONS IN CAROTID ARTERY (PLIC) STUDY.

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The carotid artery intima-media thickness (IMT), as measured non invasively by high-resolution B-mode ultrasonography, represents a surrogate index of coronary atherosclerosis. Plasma remnants lipoproteins (RLPs) are non-HDL particles, all containing apoB48, that are highly atherogenic.

The PLIC study is an epidemiological and prospective project to verify the presence and progression of atherosclerotic lesions in common carotid artery in a cohort of the Northern of Milan, and aimed to study the relation of IMT with the major risk factors for cardiovascular diseases (CVD) such as lipid parameters, lifestyle habits, and other new markers for atherosclerosis.

Subjects: from total population (n=2143), we extrapolated randomly 355 individuals free from liver disease, kidney disease, thyroid dysfunction, and not being treated with hypolipemic drugs. These subjects (40.1% men, 59.9% women) were 54.1±0.6 years old and presented with a BMI of 26.2±0.2 Kg/m^2, a LDL-C 148.1±0.3 mg/dL, HDL-C 55.2±0.8 mg/dL, triglycerides 105.8±3.8 mg/dL, plasma glucose 90.2±0.8 mg/dL, blood pressure 132/83±0.7 mmHg. IMT of carotid artery was 0.657±0.009 mm, RLP 16.8±0.7 mg/dL (all data are mean ± SE). Physical and biochemical parameters did not differ from the PLIC population.

Results: in this cohort, the prevalence of metabolic syndrome (MS, NCEP criteria), was 13.5% (27 women and 20 men). MS subjects showed higher RLPs levels (29.4±2.8 mg/dL vs 14.7±0.6 mg/dL, p<0.0001) and IMT (0.729±0.024 mm vs 0.646±0.009 mm, p<0.001) compared to the other individuals. The relationship between RLPs and the number of determinants for MS was linear (p for trend<0.0001 between RLPs and risk factors). IMT correlated linearly with the number of risk factors (p for trend<0.0001 between IMT and number of determinants). The correlation faded away in MS patients.

Conclusions: IMT and RLPs are increased in subjects with metabolic syndrome proportionally with the number of risk factors. A strong correlation between RLP and IMT is present in the population but not in MS subjects. RLPs levels in MS subjects may represent an independent risk factors for atherosclerosis.

DECREASE IN ON-TREATMENT TRIGLYCERIDE LEVEL IS DIRECTLY RELATED TO SUBSEQUENT RISK REDUCTION OF RECURRENT CORONARY EVENTS.

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Background: Fibrates were reported to be effective in reducing recurrent events in coronary heart disease (CHD) patients with elevated triglyceride. It is unknown whether this effect is related to the extent of triglyceride reduction. ***Methods and Results***: In the 3090 CHD patients enrolled in the BIP study, reduction in triglyceride serum level was evident among patients allocated to bezafibrate in a dose response manner. The extent of triglyceride reduction was significantly associated with the reduction of risk. The rates of primary endpoint by baseline triglyceride levels and tertiles of triglyceride change on bezafibrate are described in the table.

	Baseline Triglyceride Levels *(mmol/l)*			
	<2.26		2.26	
	N	n (%)	N	n (%)
Placebo	1278	172 (13.5)	220	40 (18.2)
Bezafibrate *by triglyceride reduction (mmol/l)*				
<0.15 decrease or increase	453	52 (11.5)	41	6 (14.6)
0.15 - 0.50 decrease	473	66 (13.9)	37	5 (13.5)
> 0.50 decrease	384	39 (11.2)	148	13 (8.8)

Adjusted hazard ratio was 0.45 (95% CI: 0.23-0.85) in treated patients with elevated baseline levels who reduced triglyceride level >0.50 mmol/l. The risk in treated counterparts achieving lower triglyceride reduction or failing to reduce triglyceride was similar to that of patients receiving placebo. ***Conclusion***: Bezafibrate treatment was associated with significant risk reduction among CHD patients with elevated triglyceride levels who substantially reduced their triglyceride level with treatment.

BEZAFIBRATES CAUSE MODERATE, REVERSIBLE IMPAIRMENT IN RENAL FUNCTION IN PATIENT WITHOUT PRIOR RENAL DISEASE

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Background/Aims: To determine whether bezafibrate have adverse effects on renal function.

Methods: (1) A 3-year retrospective survey of 526 patients who were on bezafibrate for a while and 614 controls following fluctuations of serum creatinine levels. (2) A prospective study on 33 patients with previous evidence of bezafibrate-induced elevation in serum creatinine. The patients were examined after 3 month on bezafibrate 400 mg/day and then after 3 month without bezafibrate. Eight patients repeated the tests after 3 months on bezafibrate 200 mg/day.

Results: Retrospective: 295 bezafibrate-treated patients (56%) and 67 controls (11%) demonstrated fluctuations ≥0.2 mg/dl in serum creatinine levels (p<0.001); 113 patients (21%) and 16 controls (3%) showed fluctuations ≥0.3 mg/dl (p<0.001). Prospective: bezafibrate 400 mg/dl increased serum creatinine from 1.16±0.19 to1.42±0.2 mg/dl (p<0.001) and urea from 37±8 to 44±8mg/dl (p<0.001); CCT decreased from 104±23ml/min to 82±27ml/min (p<0.001). CPK increased from 82±32 to 130 ±58mg/dl (p<0.0001) and urinary myoglobin increased from 95.4±21 to 199±99 mg/dl (p<0.0001). The 8 patients given bezafibrate 200 mg/dl experienced similar dose-dependent changes.

Conclusions: Bezafibrate causes quiet common, dose-dependent and reversible changes in serum creatinine in patients with normal renal function, associated with a significant increase in serum CPK and urine myoglobin, suggestive of drug-induced mild subclinical skeletal muscle injury compromising renal function.

USING GENE MARKING TO EASE REGULATION OF STEM CELL TRANSPLANTS FOR HEART DISORDERS

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While clinical efficacy is the ultimate determinant of whether stem cell transplants for heart disease will ever be approved, better understanding of the exact cellular product needed and the mechanisms of action will ease the path. Initial concepts of transdifferentiation have been compromised by animal experiments showing no evidence for this process. Other groups have claimed cell fusion may be of greater importance. Multiple other mechanisms may contribute, including development of neovasculature, provision of a scaffold to improve tissue repair and supply of cellular growth factors. Since multiple mechanisms may contribute, multiple cell types in marrow may be responsible. One way of analyzing the events is to perform experiments with different subpopulations of marrow cells but this would require vast numbers of patients, would not allow important interactions to be observed and would still allow questions to arise about the origin of the cells in the repair site.

Gene marking with retroviral vectors, which has been safely used in over 100 patients for more than 10 years may afford an opportunity to address these questions. Concerns about retroviral induced oncogenesis which had severely inhibited the field, have now been largely overcome, with a better understanding of the mechanisms involved and the development of improved vectors. By using closely related but distinctive vectors, it is possible to mark a multiplicity of different subpopulations of marrow and track the destination and fate of each subpopulation. Moreover, improved methods of analyzing vector integration sites allows assessment of the number of clones contributing to repair and can provide evidence for the development of multiple cell types in the heart from a single common precursor. The potential for safely analyzing the cellular and molecular underpinnings of this approach in humans should rapidly allow the technique to be optimized.

HEART INFARCT IN NOD-SCID MICE: THERAPEUTIC VASCULOGENESIS BY TRANSPLANTATION OF HUMAN CD34$^+$ CELLS.

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Hematopoietic (Hem) and endothelial (End) lineages derive from a common progenitor cell, the hemangioblast: specifically, the human cord blood (CB) CD34$^+$KDR$^+$ cell fraction comprises primitive Hem and End cells, as well as hemangioblasts. In humans, the potential therapeutical role of Hem and End progenitors in ischemic heart disease is subject to intense investigation. Particularly, the contribution of these cells to angiogenesis and cardiomyogenesis in myocardial ischemia is not well established. In our studies we induced myocardial infarct (MI) in the immunocompromised NOD-SCID mouse model, and monitored the effects of myocardial transplantion of human CB CD34$^+$ cells on cardiac function. Specifically, we compared the therapeutic effect of unseparated CD34$^+$ cells versus PBS and mononuclear cells (MNCs); moreover, we compared the action of the CD34$^+$KDR$^+$ cell subfraction versus the CD34$^+$KDR$^-$ subset. CD34$^+$ cells significantly improve cardiac function after MI, as compared to PBS/MNCs. Similar beneficial actions were obtained using a 2-log lower number of CD34$^+$KDR$^+$ cells, while the same number of CD34$^+$KDR$^-$ cells did not have any effects. The beneficial effect of CD34$^+$KDR$^+$ cells may mostly be ascribed to their notable resistance to apoptosis and to their angiogenic action, since cardiomyogenesis was limited. Altogether, our results indicate that, within the CD34$^+$ cell population, the CD34$^+$KDR$^+$ fraction is responsible for the improvement in cardiac hemodynamics and hence represents the candidate active CD34$^+$ cell subset.

ROLE OF APOA-I$_{Wild\text{-}Type}$ AND APOA-I$_{Milano}$ ON REVERSE CHOLESTEROL TRANSPORT: STUDIES WITH GENE TARGETING REPLACEMENT MOUSE MODEL

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Several epidemiological studies have shown that there is an independent and inverse relationship between circulating high-density lipoprotein (HDL)-cholesterol levels and coronary heart disease. Apolipoprotein A-I$_{Milano}$ (apoA-I$_M$), the first described mutant of apoA-I, is clinically associated to reduced HDL-cholesterol levels, but also to an apparent protection from premature vascular disease in the carriers. Transgenic and gene targeting replacement (gene k-in) mouse models for wild-type apoA-I (apoA-I$_{wt}$) and for apoA-I$_M$ have been developed. These have confirmed the phenotypic expression of low HDL-cholesterol, further indicating that the hypoalphalipoproteinemia is secondary to a lower liver production of the mutant. Experimental data and clinical evidences showed a great capacity of A-I$_M$ liposomes to directly remove cholesterol from arterial lesions. Aim of this study was to compare the efficiency of apoA-I$_M$ and apoA-I$_{wt}$ in promoting cholesterol transport to the liver and fecal excretion, in a k-in mouse model. A-I$_M$ k-in mice, when challenged by a high cholesterol/high fat regimen, responded both with increased lipidemia and HDL cholesterolemia, differently from what observed in apoA-I$_{wt}$ k-in line, suggesting an enhanced sensitivity to the dietary induction. Moreover, A-I$_M$ k-in mice had also reduced bile acid and sterol liver secretion. On the other hand, no differences were detected in the neutral sterol and bile acid fecal output between the two mouse lines, indicating that the overall capacity for cholesterol disposal in A-I$_M$ k-in mice is essentially similar to that measured in apoA-I$_{wt}$ expressing mice.

AAV MEDIATED LPL GENE DELIVERY IN APOE3-LEIDEN TRANSGENIC MICE

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Background. Lipoprotein lipase (LPL) gene therapy by means of intramuscular (IM) application of adeno-associated virus serotype 1 (AAV1) induces life-long normalisation of triglyceride levels in LPL deficient mice. In addition, others have shown that overexpression of human LPL in skeletal muscle of transgenic mice has a beneficial effect in mouse models for type II diabetes, obesitas and atherosclerosis. This study investigates the effects of AAV1 with LPL^{S447X} as transgene on diet-induced hyperlipidemia in male APOE3-Leiden transgenic mice. **Methods.** For this purpose we injected 6 mice with 1x10^{13} gc's/kg AAV1-LPL^{S447X} and 6 mice with PBS (IM;4 sites). One week later the mice were started on a Western type diet. At 28 weeks after starting the diet, the mice were sacrificed. **Results.** The mice that received LPL gene therapy showed a gradual increase in post-heparin LPL concentrations up to 300 ng/ml at 25 weeks after injection, but total post-heparin lipase activity was not significantly changed. In addition, we did not find effects on plasma TG, HDL-c and TC after 4 hrs fasting. Despite this, LPL gene therapy proved effective in that the treated mice displayed a faster clearance of TG and FFA after a bolus of intravenously administered Intralipid ($p<0.05$). Other interesting findings include a significantly reduced gain of weight of the AAV1-LPL^{S447X} treated animals compared to the controls ($p<0.05$), an effect that could not be attributed to a difference in abdominal fat mass. Second, muscle homogenates of the AAV1-LPL^{S447X} treated mice indicated significantly increased triglyceride content ($p<0.05$) while liver homogenates indicated significantly decreased triglyceride content ($p<0.05$) compared to controls. **Conclusions.** These data indicate that in absence of an effect on fasting lipids, expression of human LPL in the mouse skeletal muscle induces a redistribution of triglycerides and a reduced gain of body weight.

ADENO-ASSOCIATED VIRUS EXPRESSION OF HUMAN APOLIPOPROTEIN AI IN MUSCLE CELLS AND TISSUE

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Human apolipoprotein AI (Apo AI) is the major protein component of high-density lipoproteins (HDL) particles that can access the arterial intima and absorb cholesterol from lipid-loaded macrophages. These cells are a component of atherosclerotic lesions and removing cholesterol from the macrophages will reduce the size and complexity of the lesions. Recent studies showed that infusion of recombinant Apo AI into patients reduces the cholesterol content of subendothelial lesions. Such findings support the expectation that increasing Apo AI will reduce the risk of or possibly reverse established atherosclerotic disease. Effective drugs that raise Apo AI do not exist. Therefore, we chose a gene therapy approach to augment the levels of Apo AI. Adeno-associated virus (AAV) has low toxicity in vivo and can express various transgenes efficiently in muscle and secrete the corresponding proteins into serum. The human Apo AI coda was fused to the CMV promoter and then inserted into a vector yielding pAAV-ApoAI. Whether the plasmid can express and secrete the protein was tested by transfecting pAAV-ApoAI into murine muscle myotubes (C2C12), human embryonic kidney (293), human liver (HepG2) and human muscle (TE671) cells. Western blot analysis of cell lysate and spent media showed that human Apo AI was expressed in all 4 cell lines and the protein was secreted into spent media. Additionally, an actin control confirmed that Apo AI in spent media was not due to cell death. Next we used pAAV-ApoAI to produce AAV-ApoAI virus for infection. AAV-ApoAI infected human muscle cells (TE671) also expressed Apo AI, thus laying the ground work for animal studies in which AAV-ApoAI or the control AAV-LacZ was injected into the hind leg muscle of C57/BL mice. The animals were killed after 2 months and the muscle harvested for analysis. RT-PCR with specific primers for human Apo AI cDNA showed that AAV-ApoAI injected muscle expressed Apo AI mRNA but control injected mice did not. In addition, we used western blot analysis of muscle lysate to show the presence of human Apo AI protein in AAV-ApoAI but not in control virus injected mice. Finally, we found increased amounts of Apo AI in the serum from AAV-ApoAI virus injected mice compared to controls. In summary, our findings show that human Apo AI cDNA can be efficiently delivered using AAV vectors into muscle cells, in vitro and in vivo.

IDENTIFICATION OF T-CELL ACTIVATOR OX40 LIGAND AS A GENE THAT DETERMINES ATHEROSCLEROSIS SUSCEPTIBILITY

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Ath1 is a quantitative trait locus (QTL) on mouse chromosome 1 that renders C57BL/6 mice susceptible and C3H/He mice resistant to diet-induced atherosclerosis[1]. The narrowed *Ath1* region encompasses 11 known genes and three of them (*Prdx6*, *Fasl* and *Ox40l*) have known functions suggesting that they participate in atherosclerosis. In previous studies[2] *Prdx6* was excluded as an *Ath1* candidate. Here we compared atherosclerotic lesions in mice deficient in either *Fasl* or *Ox40l* with their respective controls. We also tested the association of SNPs in and around the human homologous genes with MI in human subjects. We found that only the *Ox40l* targeted mutant mice differed in the sizes of the atherosclerotic lesions with their controls. In addition, OX40L was expressed in mouse atherosclerotic lesions. Moreover, one SNP in *OX40L* was associated with the incidence of MI, and the haplotypes of *OX40L* containing this SNP were associated with the incidence of MI and the severity of coronary artery stenosis in MI patients.
OX40 ligand (OX40L) is expressed on ECs, lymphocytes, macrophages and SMCs. It generates costimulatory signals by interacting with OX40 on T lymphocytes, and enhances the proliferation and differentiation of T lymphocytes. Because many *in vivo* studies suggest that T lymphocytes promote atherosclerosis, OX40L, by enhancing T cell functions, might be proatherogenic.
With this work first we provide strong evidence that *Ox40l* underlies *Ath1*, and that polymorphisms of *OX40L* may contribute to the development of coronary artery disease in humans; second we show that it is possible to use data from a mouse QTL model to positionally identify candidate genes in a human context; third our findings suggest that the OX40L/OX40 pathway may be an excellent target for atherosclerosis therapies. The expression of OX40L in all the major cell types in atherosclerotic lesions suggests that it plays a central role in atherosclerotic lesions and since both OX40L and OX40 are expressed more in inflammatory than in normal tissues, targeting the OX40L/OX40 pathway may inhibit local inflammation without risks for the immunosystem.
[1]Paigen et al. PNAS USA 1987;84:3763-7.
[2]Phelan et al. Free Radic Biol Med 2003;35:1110-20.

EVALUATION OF ISIS 301012, AN ANTISENSE INHIBITOR TO HUMAN apoB-100, IN HEALTHY VOLUNTEERS.

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ISIS 301012 is an antisense compound designed to specifically inhibit the expression of human apoB-100. In man, apoB-100 plays an essential role in the regulation of cholesterol biosynthesis and constitutes the principal apolipoprotein present within triglyceride-rich, VLDL and LDL-cholesterol (LDL-C). ApoB-100 is synthesized predominantly in the liver of non-human primates and man. The site on which ISIS 301012 hybridizes is homologous to the cynomolgus monkey mRNA with the exception of two base mismatches. ISIS 301012 has effectively reduced apoB-100 mRNA and protein in both human and monkey hepatocytes *in vitro*. ISIS 301012 was also tested *in vivo* utilizing transgenic mice which expressed the human apoB-100 gene. Following intraperitoneal administration to these mice, ISIS 301012 effectively reduced both hepatic and serum apoB-100 by 80%, with reductions sustained over three months. Based upon these results, ISIS 301012 was administered to lean cynomologus monkeys and produced a dose-dependent reduction in hepatic apoB-100 mRNA and significantly reduced LDL-C (16%) after 4 weeks of treatment. Following three months of treatment, no evidence of hepatic steatosis or interaction with the cytochrome p450 system was observed. ISIS 301012 is currently being evaluated in a double-blind, placebo-controlled, dose escalation study to evaluate dose levels of 50 to 400 mg ISIS 301012 or placebo per week on total cholesterol, LDL-C, VLDL-C, and apoB-100 in healthy subjects with fasting total cholesterol of 200-300 mg/dL and a BMI $\leq$ 30 kg/m^2. Results from this study may demonstrate that an antisense inhibitor targeting apoB-100 can reduce apoB-100, LDL-C and total cholesterol. Preliminary results from the clinical study will be presented at this meeting.

GUIDELINES AND TARGETS OF LIPID-LOWERING THERAPY: THE CURRENT USA PERSPECTIVE

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The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program (NCEP) issued guidelines on cholesterol management in 2001. Subsequently, 5 clinical trials of statin therapy with clinical endpoints have been published. NCEP reviewed the results of these recent trials, and updated ATP III guidelines. LDL-cholesterol (LDL-C) remained the primary target of lipid-lowering therapy. These trials support the ATP III treatment goal of LDL-C < 100 mg/dL but also, add to growing evidence for the concept that "the lower, the better" for LDL-C in high-risk patients. Importantly, the NCEP update affirms that therapeutic lifestyle changes (TLC) are essential for maximal risk reduction. The recent trials give added support for including patients with diabetes in the high-risk category. According to the update, when risk is *very high*, an LDL-C goal of < 70 mg/dL is a therapeutic option, i.e., a reasonable clinical strategy, based on available clinical trial evidence. Very high risk patients are those with established CVD plus (a) multiple major risk factors (especially diabetes), (b) severe and poorly controlled risk factors (especially continued cigarette smoking), (c) multiple risk factors of the metabolic syndrome (especially high triglyceride $\geq$ 200 mg/dL plus non-HDL-C $\geq$ 130 mg/dL with low HDL-C [< 40 mg/dL]) and (d) those with acute coronary syndromes. For other *high-risk patients,* the LDL-C goal remains < 100 mg/dL. For *moderately high-risk persons* (2+ risk factors and 10-year risk 10-20%), the recommended LDL-C goal is < 130 mg/dL; but an LDL-C goal <100 mg/dL is a therapeutic option based on recent trial evidence. When LDL-lowering drug therapy is employed in high-risk or moderately high-risk persons, it is advised that intensity of therapy be sufficient to achieve at least a 30-40% reduction in LDL-C levels. Finally, for people in lower-risk categories, recent clinical trials do not modify the goals and cutpoints of therapy.

GUIDELINES AND TARGETS OF LIPID LOWERING THERAPY. A EUROPEAN VIEW.

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Since 1994, European recommendations for prevention of coronary artery disease have been developed in a collaboration between the major scientic societies for atherosclerosis, cardiology and hypertension, later joined by societies for diabetes, general practice, behavioral medicine, and the major European heart foundations. This ecumenical approach emphasizes interaction between risk factors rather than particular risk factors, and guidelines for dealing with each risk factor are therefore fairly simple. For example, goals of lipid lowering are total cholesterol < 5 mmoles/l and LDL-cholesterol < 3 mmoles/l for most people and < 4.5 and < 2.5 mmoles/l, respectively, for high-risk people. There are arguments for still lower goals, but they would result in even greater medicalization of prevention programs, unless the societal determinants of the disease, e.g. agricultural and food policies, were more adequately addressed. The risk function used in the 1994 and 1998 versions of the guidelines was based on Framingham data, but European data (SCORE) enabled us in 2003 to take into account the substantial gradients of cardiovascular risk across the continent. Since 1994 the European recommendations have encouraged adaptation to national needs. In wealthy countries with low disease prevalence, expensive drugs for everyone is economically realistic when 10 risk of cardiovascular death exceeds 5% (~ 20% risk of coronary event). In countries with less money and more disease, higher levels of 10 year risk may be necessary before physicans prescribe drugs to lower lipids, blood pressure or glucose. Guidelines, written to aid physicians in their clinical work, can less appropriately be used for administrative and even medico-legal purposes, and Europeans have begun to understand the importance of the nature and limitations of evidence-based medicine and evidence-based guidelines.

THE VIEW OF THE INTERNATIONAL TASKFORCE FOR PREVENTION OF CORONARY HEART DISEASE

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A consensus is emerging that the best way to initially allot patients to risk factor categories is by means of risk algorithms or scores that take account of the totality of the major risk factors present. Such algorithms have been derived from the Framingham study in the United States and from the Prospective Cardiovascular Münster Study in Germany. Ideally, one should use a risk algorithm derived in the population to which the patient belongs. In practise, this is not always possible, so that algorithms or scores need to be calibrated for use in populations other than those in which they were derived.
A major challenge today is how to further stratify risk after use of a classical risk score. This problem is particularly acute for the 15% of patients at intermediate risk of coronary heart disease (i.e. who have a 10-year CHD risk of 10 to 20%). About 40% of all myocardial infarctions occur in this intermediate risk group.
The view of the International Task Force is that further stratification in such patients is best achieved by the use of emerging risk factors. At present, most evidence exists for the five emerging risk factors in the following table.

Emerging risk factors for coronary artery disease	
A	Evidence of atherosclerosis on non-invasive imaging, i.e. an age- and sex-adjusted calcium score of the coronary arteries above the 75th percentile or an increased intima-media thickness ratio.
B	Lipoprotein (a) 30 mg/dL.
C	C-reactive peptide > 3 mg/L in the absence of acute inflammation.
D	Homocysteine 12 µmol/L.
E	4 of the 10 genetic risk factors in Table 3, particularly in patients with a positive family history of coronary heart disease.

The presence of emerging risk factor A and/or two or more of the emerging risk factors B to E may tip the balance towards classification of patients in the intermediate or high risk categories into the next highest category, i.e. patients at intermediate risk are classified as being at high risk, and patients at high risk are classified as being at very high risk. In patients at low or moderate risk of CHD (10% CHD risk in 10 years), use of emerging risk factors is not required.
Use of emerging risk factors may alter treatment targets. Thus, in patients at very high risk of CHD, there is a case to made for lowering LDL cholesterol to well below 100 mg/dL (2.6 mmol/L), for example to 70 mg/dL (1.8 mmol/L).

THE CURRENT ASIAN-PACIFIC PERSPECTIVE

Japanese Guideline for the Management of Atherosclerotic disease and the Asian Definition of Metabolic Syndrome and its Molecular Mechanism.
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Atherosclerotic diseases have become crucial problem recently in Japan and Asian countries as so in western countries, because cardiovascular death contributes to 30% of cause of death in recent years according to the reports from National Health and Welfare Ministry. The importance of hypercholesterolemia has been recognized not only by lipidologists but also general physicians in these ten years. Japanese Atherosclerosis Society set up the first guideline for the treatment of hyperlipidemia in 1997 in which cutoff point of plasma cholesterol, triglyceride and HDL-cholesterol were recommended to be 220 mg/dl, 150 mg/dl and 40 mg/dl respectively. In this guideline, although the importance of other risk factors was considered, target cholesterol levels after treatment was set only in three categories such as the case without any risk factor, with any risk factors and with already coronary artery disease. In 2002, Japanese Society renewed it as the guideline for the management of atherosclerotic diseases which was not limited to that for the management of hyperlipidemia. In this guideline, LDL-cholesterol instead of total cholesterol is highlighted as a marker for the management of hypercholesterolemic subjects. Subjects are divided into 6 categories which consist of the hypercholesterolemic subjects without any other risk factor as Category A, the subjects with other risk factors as Category B (which are further divided into Category B1, B2, B3 and B4 according to number of other risk factors) and the subjects with coronary artery disease as Category C. Treatment target for LDL-cholesterol are set to be 160 mg/dl for Category A, 140 mg/dl for category B1and B2, 120 mg/dl for Category B3 and B4 and 100 mg/ml for Category C.

In the new guideline, the importance of multiple risk factor clustering syndrome, so-called metabolic syndrome is emphasized on the basis of several reports showing magnitude of multiple risk factors for ischemic heart disease. For example, a case control study in Japanese employee supported by National Labor Ministry demonstrated that the subjects with the combination of 3 or more risk factors among obesity, hypertriglyceridemia, hyperglycemia and hypertension, have the increased relative risk to 35.8 times compared the subjects without any risk factor..

In this lecture, I would like to show the definition and the diagnostic criteria of metabolic syndrome in Japanese and discuss the difference from those of western countries. In addition, I would like to discuss molecular mechanism from our studies on adipocytes biology that multiple risk factors cluster in one individual and also this syndrome is so atherogenic.

NON-INVASIVE METHODOLOGY: INTIMA MEDIA THICKNESS

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The intima-media thickness (IMT) of carotid arteries, measured by high-resolution B-mode ultrasound, is one of the methods of choice for determining the presence and the extent of atherosclerosis. Starting from the initial studies showing a significant correlation with pathohistologic measurements this variable has been widely used to investigate its correlation with vascular risk factors (conventional and not conventional) as well as to investigate the association with the extent of atherosclerosis and end-organ damage of high-risk patients. IMT is now widely accepted as a measure of the atherosclerotic burden and as a predictor of subsequent vascular events. By using specific softwares, which allow the automatic edge detection of the blood-intima and media-adventia interfaces, it is now possible to measure this ultrasonic variable with a very high reproducibility (absolute differences between replicate scans not > 12-15 µm). Because of its quantitative nature, carotid IMT (C-IMT) is frequently used in clinical trials also to test the efficacy of therapeutic intervention and a variety of drugs, with different mechanism of action, have been shown to influence C-IMT. In some studies the pharmacologically-induced changes in C-IMT have been also associated with a concomitant reduction of cardiovascular events, which further supports the concept that IMT can be considered as a surrogate index of atherosclerosis. The translation of the concept of C-IMT from biomarker to that of surrogate end point, however, requires additional evidence showing that, like cross-sectionally assessed IMT, also C-IMT progression is an effective predictor of new vascular events. Studies, however, aimed at evaluating the relationship between C-IMT progression and cardiovascular events are still required. To this end an observational study, aimed at defining the predictive properties of IMT progression of future cardiovascular events, has been designed. The IMPROVE study is an European, multicentre, longitudinal (three year) ongoing study performed in a population of 3600 patients at high risk of cardiovascular events. The results of this study are expected by 2007.

CORONARY PLAQUE IMAGING WITH CT TECHNIQUES

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Modern CT technology, especially the use of multi-slice spiral CT (MSCT) scanners with retrospectively ECG-gated image reconstruction, permits visualization of the coronary arteries.
The presence and amount of coronary calcium can be deomstrated in native CT scans. Coronary calcium been shown to correlate to the presence and extent of coronary atherosclerotic plaque. Consequently, it has been possible to demonstrate that coronary calcium in asymptomatic individuals has prognostic significance concerning the occurence of coronary artery disease events. The prognostic value of coronary calcium, which constitutes direct evidence of atherosclerotic plaque, is independent from and higher than that of traditional risk factors. Even though the prognostic value is undisputed, it has not been clarified yet which individuals profit from performing coronary calcium imaging by CT as part of their risk assessment. Furthermore, coronary calcium has been shown to be progressive over time. The degree of progression can be influenced by lipid-lowering therapy. Even though the concept of repeated calcium imaging as a tool to assess the effectiveness of lipid-lowering therapy is intriguing, it has not been validated yet.
Visualization of coronary atherosclerotic plaque - calcified and non-calcified - after injection of contrast is not yet sufficiently validated for clinical applications. A rough estimation of plaque burden seems possible, but no follow-up studies to investigate changes over the course of time have been conducted so far. Similarly, promising initial results have been obtained concerning the characterization of plaque composition by CT, but clinical applications will require further validation
In summary, MSCT is a promising tool for the non-invasive assessment of coronary atherosclerotic plaque. Clinical applications seem most likely for coronary calcium imaging, while visualization and characterization of non-calcified plaque is not backed by sufficient clinical data yet.

NONINVASIVE IMAGING BY MRI.

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Atherosclerosis and its thrombotic complications are the major cause of morbidity and mortality in the industrialized countries. Despite advances in our understanding of the pathogenetic mechanisms and new treatment modalities, the absence of an adequate noninvasive method for early detection limits prevention or treatment of patients with various degrees and localizations of athero-thrombotic disease.
The ideal clinical imaging modality for atherosclerosis should be safe, inexpensive, noninvasive or minimally invasive, accurate and reproducible, thus allowing longitudinal studies in the same patients. Additionally, the results should correlate with the extent of atherosclerotic disease and have high predictive values for clinical events.
In vivo, high-resolution magnetic resonance imaging (MRI) has recently emerged as one of the most promising techniques for the noninvasive study of athero-thrombotic disease in several vascular beds such as the aorta, the carotid arteries and the coronary arteries. Most importantly MRI can be used to characterize plaque composition as it allows the discrimination of lipid core, fibrosis, calcification, and intra-plaque hemorrhage deposits. MRI findings have been extensively validated against pathology in ex vivo studies of carotid, aortic, and coronary artery specimens obtained at autopsy and using experimental models of atherosclerosis. In vivo, MRI of carotid arteries of patients referred for endarterectomy has shown a high correlation with pathology and with previous ex vivo results. A recent study in patients with plaques in the thoracic aorta showed that, compared with transesophageal echocardiography, plaque composition and size are more accurately characterized and measured using in vivo MRI. The composition rather than the degree of stenosis determines the patient outcome. Therefore, a reliable noninvasive imaging tool able to detect early atherosclerotic disease in the various regions and identify the plaque composition is clinically desirable.
MRI has potential in the detection arterial thrombi and in the definition of thrombus age. MRI has been used to monitor plaque progression and regression in several animal model of atherosclerosis and in human. Thus, MRI opens new strategies ranging from screening of high-risk patients for early detection and treatment as well as monitoring the target areas for pharmacological intervention.

INTRAVASCULAR ULTRASOUND: INSIGHTS INTO ATHEROSCLEROSIS

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Atherosclerosis imaging has taken on increasing importance in the understanding of the natural history of coronary artery disease and the processes leading to luminal narrowing, as well as the assessment of disease burden and therapy efficacy. Intravascular ultrasound (IVUS) has emerged as the new gold standard for atherosclerosis imaging because it provides cross-sectional images of both the arterial wall and lumen with excellent resolution, reveals the diffuse nature of atherosclerosis and the involvement of reference segments and takes into account vessel wall remodeling. In addition to its clinical indications, IVUS is now widely used as the primary efficacy assessment measure of several anti-atherosclerotic approaches in randomized clinical trials. Advantages of IVUS include its ability to reveal anti-atherosclerotic effects within a relatively short period of time and with a reasonable sample size, in contrast to trials assessing angiographic changes or clinical events. IVUS can also help to determine dose-response relationships in the development of novel pharmacologic agents. IVUS is currently the ideal imaging modality for clinical trials of atherosclerosis progression/ regression.

SHOULD HDL OR TRIGLYCERIDES BE THE TARGET IN VASCULAR DISEASE PREVENTION?

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The metabolic interrelationship between HDL cholesterol and triglyceride rich lipoproteins (TRL) continues to generate debate regarding the relative importance of these entities in the etiology of vascular disease. Their statistical linkage in community based studies as well as clinical trial data have done little to resolve this issue. Triglyceride rich lipoproteins (TRL) are complex in structure and dynamic in form as they circulate through the plasma compartment. The concentrations and the composition of TRL are sensitive to the metabolic state of the individual and are therefore volatile, causing difficulty in relating plasma concentrations to outcome measures. HDL concentrations are more stable and this may explain in part the stronger correlations that have been drawn between this lipoprotein and vascular endpoints.
The concept of remnants of TRL as a major etiologic agent may explain why extremely high triglyceride concentrations as in lipoprotein lipase deficiency do not lead to early arteriosclerosis. This also may explain why the relationship between TRL and vascular endpoints is most clearly demonstrable over the range of fasting triglyceride concentrations from 50 to 400 mg/dl (0.57 to 4.55 mmol/L). Higher concentrations do not confer significantly higher risk.
The relationship between vascular disease and TRL is particularly strong in women. Current studies in our laboratory have found TRL to be a predictor of reduced vascular compliance in the arteries of the lower extremities of women that far exceeds relationships with any other characteristic of lipoproteins.
To date, the use of drugs to lower triglycerides has not resolved the issue since the consequence of such treatment also involves the elevation of HDL-C. It is also interesting that HDL raising fibrates seem to work uniquely well in patients with higher triglycerides and insulin resistance. The development of new drug classes (i.e. CETP inhibitors) that increase HDL by inhibiting the transfer of cholesterol from these lipoproteins into TRL offers a new opportunity to test the concept that simply having more HDL in the plasma will provide for increased transport from peripheral tissues to the liver. In theory, this will reduce the accumulation of cholesterol in the artery wall and reduce vascular disease. Even this does not remove the confusion since the inhibition of CETP also reduces cholesterol content of TRL.
Greater knowledge of the function of apolipoprotein metabolism, cell surface receptors and lipid transport systems in membranes will be required before more specific targets can be chosen to allow greater insight into the atherogenic mechanisms that can be effectively modified.

HDL MIMETICS FOR CARDIOPROTECTION AGAINST ISCHEMIA/REPERFUSION INJURY

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A low plasma HDL cholesterol has been associated with a high risk of ischemic heart disease and with unfavorable prognosis after an acute ischemic event. This latter association may reflect either accelerated atherogenesis, or a direct detrimental effect of the low HDL level on post-ischemic cardiac function. We recently showed a direct cardioprotective effect of plasma-derived human HDL against ischemia/reperfusion (I/R) injury, which is mediated through a reduced cardiac tumor necrosis factor-α (TNFα) content and an enhanced cardiac prostaglandin release. Most of plasma HDL functions can be mimicked by well defined, apolipoprotein-specific synthetic HDL (sHDL) made of phosphatidylcholine and apoA-I, the major lipid and protein constituents of plasma HDL. These sHDL proved effective in causing the regression of atherosclerotic disease in coronary patients, and were tested for direct cardioprotection in isolated rat hearts subjected to I/R injury. When administered immediately before ischemia, sHDL caused a rapid, dose-dependent improvement of post-ischemic cardiac function, associated with a reduced cardiomyocyte damage. sHDL administered in the first minutes post-ischemia also exerted a significant protection against I/R-induced cardiac dysfunction, but cardioprotection was higher when sHDL were given pre-ischemia. The sHDL cardioprotection was mediated through the reduction of cardiac TNFα expression and content, an enhanced cardiac prostaglandin production, and the prevention of cardiac MMP-2 release. The present experimental data indicate that sHDL may become a clinically useful form of treatment in situations in which myocardial I/R occurs, such as acute coronary syndromes, cardiac surgery and transplantation, or revascularization with angioplasty, thrombolysis, or bypass surgery.

APOLIPOPROTEIN A-I AND THE MOLECULAR VARIANT A-I$_M$: EVALUATION OF EFFECTS ON ATHEROSCLEROSIS IN A KNOCK-IN MOUSE MODEL

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Apolipoprotein A-I$_M$ (apoA-I$_M$) (R173C) has been the first identified molecular variant of human apoAI. Individuals heterozygous for this mutant allele are characterized by hypoalphalipoproteinemia not associated with an increased risk of coronary artery disease. In vitro assays have suggested an increased efficiency of this mutant, compared to the normal apoA-I, in protecting from the development of atherosclerosis. In order to directly compare in vivo these two forms of apoA-I, previously generated A-I or A-I$_M$ knock-in lines were crossed with human apoB/A-II expressing mice to obtain mouse lines (h-B/A-II/A-I$_M^{Hu/Hu}$ and h-B/A-II/A-I$^{Hu/Hu}$, respectively) with a genetic background susceptible to diet-induced atherosclerosis. The h-B/A-II/A-I$_M^{Hu/Hu}$ had a pre-diet high-risk lipid profile compared to the line expressing apoA-I, i. e. characterized by low HDL cholesterol levels and by elevated plasma levels of triglycerides. Ten mice of both sexes and lines, aged between 8 and 10 weeks, were placed on atherogenic diet for 18 weeks. A gender difference in response to high fat diet was observed in both h-B/A-II/A-I$_M^{Hu/Hu}$ and h-B/A-II/A-I$^{Hu/Hu}$ mice, i.e. females of both mouse lines had higher total cholesterol levels compared to males, and h-B/A-II/A-I$_M^{Hu/Hu}$ females were characterized by higher human apoB and lower HDL cholesterol levels compared to h-B/A-II/A-I$_M^{Hu/Hu}$ males. Mean lesion area in male mice was comparable between the two mouse lines. On the contrary, the mean lesion area measured in h-B/A-II/A-I$_M^{Hu/Hu}$ females was greater than that observed in h-B/A-II/A-I$^{Hu/Hu}$ females.

HIGH DENSITY LIPOPROTEINS ATTENUATE ENDOTHELIAL CELL ACTIVATION BY INHIBITING CYTOKINE-INDUCED INTERLEUKIN-6 PRODUCTION

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Objective – The hypothesis that HDL may influence arterial wall pathology by inhibiting the cytokine-induced production of interleukin 6 (IL-6) by endothelial cells was investigated *in vitro* and *in vivo*.
Methods and Results – Human umbilical vein endothelial cells (HUVEC) were treated with human plasma HDL and stimulated with TNFα; the supernatant IL-6 concentration was then measured. HDL caused a concentration-dependent inhibition of TNFα-induced production of IL-6; at the maximum tested concentration (2 mg of protein/ml), HDL decreased IL-6 production by 58.5±1.5%. The effect of human plasma HDL on IL-6 mRNA levels was investigated by RT-PCR analysis. TNFα induced an increase in IL-6 mRNA, which was markedly reduced by HDL treatment. TNFα-induced IL-6 production was completely blocked by SB203580, a selective p38 mitogen-activated protein kinase (MAPK) inhibitor, while it was not affected by the inhibition of ERK1/2 and JNK pathways; HDL were shown to decrease TNFα-induced p38 MAPK phosphorilation. Reconstituted HDL made with apolipoprotein A-I and phosphatidylcholine were as effective as plasma HDL, while the single components were ineffective. To investigate whether the inhibitory effect of HDL on IL-6 production in cultured HUVEC may have clinical significance, plasma IL-6 concentration was measured in individuals with low, average, or high plasma HDL-C levels. The median plasma IL-6 concentration was significantly higher in subjects with low HDL (2.54 pg/ml) compared with those with average or high HDL (1.31 pg/ml and 1.47 pg/ml, respectively). When all subjects were considered together, a lower HDL-C was the strongest independent predictor of higher IL-6 ($F = 25.38$, $P < 0.001$).
Conclusion - By inhibiting IL-6 production and lowering plasma IL-6 concentration, HDL may limit the pro-atherogenic effects of both acute and chronic inflammatory states, of which IL-6 is a key orchestrator.

DESIGN OF NOVEL ANTI-ATHEROSCLEROTIC APOLIPOPROTEIN A-I ANALOGUES/HDL MIMICS WITH INCREASED PLASMA LIFE TIME

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An increased level of plasma high-density lipoprotein (HDL) is the aim of several therapeutic strategies for combating cardiovascular disease. To evaluate the role of the kidney we investigated the renal handling of HDL in patients with renal proximal tubular re-absorption failure. A greatly increased urinary excretion of apoA-I was found, whereas excretion of phospholipids, triglycerides, cholesterol and cholesterol esters was low. This excretion pattern strongly indicates that the kidney functions as an important clearance organ for apoA-I molecules released from or never incorporated in the HDL particle. That size determines apoA-I plasma lifetime was further indicated by a prolonged plasma lifetime in mice of a cholesterol efflux-promoting trimerized apoA-I analogue. Furthermore, high-dose injection of the the apoA-I trimer showed significant reduction in progression of in aortic root atherosclerosis of low-density lipoprotein receptor-deficient mice. Modulation of the renal clearance of endogenous apoA-I or direct administration of exogenous apoA-I analogues with retarded clearance might therefore represent novel approaches for treatment of atherosclerosis.