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NEW INSIGHTS INTO LIPOPROTEIN METABOLISM AND TARGETS FOR THE TREATMENT OF CARDIOVASCULAR DISEASE

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High-density lipoproteins (HDL) are an independent risk factor for the development of cardiovascular disease (CVD). HDL protects against CVD by four major mechanisms: 1. Reverse cholesterol transport – a process whereby HDL transports excess cholesterol from peripheral cells to the liver for removal from the body by biliary secretion of cholesterol or bile acids; 2. Protection of low density lipoproteins (LDL) from oxidation; 3. Anti-inflammatory protection due to inhibition of expression of adhesion molecules on endothelial cells, preventing monocyte movement into the vessel wall; and 4. Modulation of vascular endothelium by regulation of nitrous oxide production.

In the reverse cholesterol transport, three pathways are involved in the efflux of cholesterol from cholesterol-loaded peripheral cells to HDL and the transport of the excess cholesterol back to the liver. These pathways reduce cellular cholesterol by: 1. The ABCA1 transporter which effluxes cholesterol to lipid-poor apoA-I to form nascent HDL, which is converted to mature HDL by esterification of cholesterol to cholesteryl esters (CE) by lecithin cholesterol acyltransferase (LCAT); 2. SR-BI, which decreases cholesterol by transfer to mature HDL; and 3. Passive diffusion which facilitates removal of excess cellular cholesterol to mature HDL and other plasma lipoproteins. Recent studies utilizing ABCA1 transgenic mice as well as ABCA1 adenovirus constructs have established that the major source of plasma HDL cholesterol as well as poorly lipidated HDL is the liver. This data is consistent with reverse reverse cholesterol transport where mature HDL and poorly lipidated apoA-I, which are synthesized by the liver, are transported to the periphery removing excess cholesterol from cells by interaction with the SR-BI receptor and ABCA1 transporter respectively, and ultimately returning the excess cholesterol back to the liver for removal from the body.

Several lines of evidence including data from apoA-I transgenic mice and rabbits, and infusions of apoA-I/phospholipids complexes in hypercholesterolemic rabbits and man indicate that raising HDL may be associated with protection against CVD. Poorly lipidated apoA-I as well as short apoA-I mimetic peptides have been shown to increase cholesterol efflux from cells in culture by the ABCA1 transporter pathway and mature HDL is effective in facilitating efflux from the SR-BI pathway in peripheral macrophages. The most promising approach to long-term therapy to raise HDL is the increase in HDL associated with administration of a CETP inhibitor. These combined results provide support for the concept that raising HDL both by acute HDL as well as long-term HDL therapy may represent a new therapeutic approach for the treatment and prevention of CVD.

GENES AND HDL HOMEOSTASIS

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Numerous studies have shown that high levels of high-density lipoprotein cholesterol (HDL-C) have a protective effect upon the development of CHD. Although environmental factors influence HDL-C, genetic factors carry a similar weight (40-60%) in the general population. A number of studies have provided evidence that HDL-C has a considerable heritable component. The complexity of HDL metabolism and of reverse cholesterol transport has resulted in a long list of genes potentially involved in the regulation of HDL-C variability. This lecture provides a comprehensive summary of genes currently known to potentially affect the variability of HDL-C levels in the general population as well as chromosomal regions that potentially contain novel genes involved in HDL-C metabolism and reverse cholesterol transport. Furthermore, reported interactions between genes and environmental factors to be taken into consideration when examining the association and/or linkage between genetic variants and HDL-C levels will be discussed.

TRANSCRIPTION FACTORS : FROM TRANSCRIPTIONAL ACTIVATION TO CLINICAL IMPLICATIONS

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The hypolipidemic fibric acid drugs are peroxisome proliferator-activated receptor alpha (PPARα) ligands. PPARα activated by fibric acids form heterodimers with the 9-cis retinoic acid receptor (RXR). The PPAR/RXR heterodimers bind to peroxisome proliferator response elements (PPRE), which are located in numerous gene promoters and increase the level of the expression of mRNSAs encoded by PPARα target genes. Fibric acids decrease triglyceride plasma levels through increases in the expression of genes involved in fatty acid-beta oxidation. Furthermore, they decrease triglycerides by increasing lipoprotein lipase gene expression and by decreasing apolipoprotein C-III gene expression. Very recently, we demonstrated that apolipoprotein A-V, a crucial determinant of plasma triglyceride levels is highly responsive to PPARα activators. Fibric acids increase high-density lipoprotein (HDL) cholesterol partly by increasing apolipoprotein A-I and apolipoprotein A-II gene expression. We reported also that fibric acids and other PPARα activators increase reverse cholesterol transport by stimulating the expression of SR-BI/CLA-I and ABCA-I which induce the efflux of excess cellular cholesterol. Fibric acids also reduce vascular inflammation and the expression of genes involved in different vascular functions (ie, vasomotricity, thrombosis). Fibric acids are used to treat primary hypertriglyceridemia and mixed hyperlipidemia. Some fibric acid molecules are active in essential hypercholesterolemia. Clinical evidence shows that fibric acids reduce coronary atherosclerosis progression in dyslipidemic patients (eg. Bezafibrate, gemfibrozil) and in type 2 diabetic patients (fenofibrate). Gemfibrozil decreases coronary morbidity and mortality in patients with low HDL cholesterol, normal triglycerides, and normal low-density lipoprotein (LDL) cholesterol plasma levels. Further clinical studies are necessary to investigate if fibric acids or other PPARα activators decrease cardiovascular mortality in type 2 diabetes and other dyslipoproteinemia.

DEFINITION AND PREVALENCE OF THE METABOLIC SYNDROME

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The metabolic syndrome represents a constellation of metabolic risk factors for atherosclerotic cardiovascular disease (ASCVD) occurring in a single individual. There are five metabolic risk factors that accompany the metabolic syndrome: atherogenic dyslipidemia (elevated apolipoprotein B, elevated triglyceride, small LDL particles, and low HDL-cholesterol), elevated blood pressure, elevated glucose, a prothrombotic state, and a proinflammatory state. The major underlying risk factors for the metabolic syndrome are obesity and insulin resistance. Other factors that can worsen the syndrome are lack of physical activity, advancing age, and hormonal factors (e.g. androgens and corticosteroids). Several different criteria have been proposed for clinical diagnosis of the metabolic syndrome. There is a large amount of overlap among these different criteria, but emphasis is different. For example, the World Health Organization criteria emphasis insulin resistance as the major underlying risk factor, whereas the USA National Cholesterol Education Program places more emphasis on obesity. Most organizations however are in agreement that ASCVD is the major clinical outcome of metabolic syndrome. There is further agreement that metabolic syndrome is a major risk factor for type 2 diabetes. In fact, a significant portion of the ASCVD that develops in patients with the metabolic syndrome occurs in persons after they have developed type 2 diabetes. The underlying risk factors, prevalence and clinical manifestations of the metabolic syndrome vary among different populations. These differences likely represent variations in genetic susceptibilities of the different populations. Regardless, the rising prevalence of obesity in the world heralds a marked increase in the prevalence of metabolic syndrome along with its major outcomes—ASCVD and type 2 diabetes.

PPAR ALPHA AGONISTS AND THE METABOLIC SYNDROME

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Peroxisome proliferator activated receptors alpha (PPARα) are transcription factors belonging to the nuclear receptor superfamily which are activated by fatty acids and their derivatives. PPARα is mainly expressed in tissues having a high metabolic rate such as liver and muscles and in the cells of the atherosclerotic lesion. PPARα regulates genes involved in lipid metabolism, haemostasis and vascular inflammation, making it a candidate gene for risk of dyslipemia, atherosclerosis and coronary artery disease.

A broad spectrum of compounds can serve as PPARα ligand and activate the receptor. After activation, PPARα binds, upon heterodimerisation with RXR, to specific response element in the promoter of target genes, thus regulating the transcription of these genes. PPARα activation have also recently been shown to modulate gene transcription by negatively interfering with other transcription factor pathways in a DNA binding-independent manner.

In human vascular cells, interference with the nuclear factor NF-κB represses cytokine-induced activation of a number of inflammatory genes such as VCAM-1, COX2, IL-6.

PPARα activators furthermore improve glucose homeostasis and influence energy metabolism by enhancing fatty acid flux and degradation in the liver. These observations indicate a modulating role for PPARα in the pathogenesis of age-related disorders predisposing to atherosclerosis.

LIPOPROTEIN METABOLISM IN THE METABOLIC SYNDROME

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The association between plasma cholesterol and cardiovascular risk is well known, but there are additional risk factors that increase this risk. The incidence of obesity, primarily visceral obesity, is increasing at an alarming rate and heralds a parallel increase in diabetes and cardiovascular disease. With visceral obesity often comes a myriad of conditions including insulin resistance, dyslipidemia, a pro-inflammatory and pro-thrombotic state, and hypertension, known collectively as the metabolic syndrome. The syndrome primarily results from environmental factors including a sedentary lifestyle and excess caloric intake. Although different definitions of the metabolic syndrome exist, elements common to all include dyslipoproteinemia, specifically high plasma triglycerides and low HDL cholesterol. Lipoprotein tracer studies have identified the metabolic abnormalities that give rise to elevated concentrations of apoB and low concentrations of HDL cholesterol. These include oversecretion of VLDL, impaired catabolism of all apoB-containing lipoproteins and, hypercatabolism of HDL apoAI, respectively. In addition to genetic factors, the distribution of body fat and concentration of adipocytokines is associated with VLDL secretion and clearance. Statins and fibrates have been used to reduce dyslipidemia in the metabolic syndrome. Statins act to improve the catabolism of the apoB-containing lipoproteins, while fibrates increase apoB catabolism, but also the conversion of VLDL to LDL, the secretion of HDL apoAI and apoAII is also increased as is the concentration of LpAI:AII particles. Neither therapy reduces VLDL secretion; however, this may be due to persistent insulin resistance. Weight reduction, in contrast, reduces VLDL secretion; the effect on HDL metabolism remains unknown. Associated with weight reduction is a reduction of visceral fat mass, improved insulin sensitivity, increased adiponectin levels and increased clearance of VLDL and LDL particles.

INSULIN RESISTANCE AND THE METABOLIC SYNDROME

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WHO and ATP III criteria differ with respect on the weight given to hyperinsulinemia / insulin resistance versus hyperglycemia/ glucose intolerance. This difference is conceptual and the cause for the ongoing debate whether insulin resistance is the unifying factor underlying the syndrome. Both insulin resistance and hyperinsulinemia predict Type 2 diabetes and are risk factors for future CVD. In this context it should be recognized that hyperinsulinemia is a compensatory phenomenon to insulin resistance. In the majority of the studies insulin resistance has been defined using surrogate measures (fasting insulin concentration, HOMA index etc). In non-diabetic subjects with the MS measures of insulin resistance correlates significantly with different components of the MS. In general the subjects with the MS are more insulin resistant and have higher levels of CVD risk factors independently of the used criteria. Growing data suggest that the MS more strongly predicts CHD and CVD as a cluster than its individual components. In the Botnia study the relative risk of CHD in non-diabetic subjects with the syndrome was 1.66 ($p= 0.002$ by WHO and 1.45 ($p= 0.03$) by ATP III criteria during the follow-up for 9.5 years. We also evaluated risk factors for the development of Type 2 diabetes in subjects followed for a median time of 6 yrs. Subjects with MS had 3.5 fold ($p <0.001$) increased risk of Type 2 diabetes the risk being similar using both the WHO and ATP III criteria. Notably a family history of diabetes together with features of the MS conferred a very high risk of Type 2 diabetes. Importantly both insulin resistance and impaired β cell function were independent predictors of diabetes. Thus the inability of the β cells to compensate for the degree of insulin resistance seems to be the key defect leading to Type 2 diabetes. In conclusion the data suggest that insulin/ insulin resistance may predict differently CVD risk and development of Type 2 diabetes.

OPTIMAL MANAGEMENT OF CVD RISK IN PATIENTS WITH THE METABOLIC SYNDROME

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The worldwide prevalence of type 2 diabetes has reached epidemic proportions and there is no evidence that this rapid growth will "plateau" in the coming years. This situation can be largely attributed to our "toxic" affluent lifestyle explaining why a growing proportion of our population is in chronic positive energy balance leading to weight gain which is a major risk factor for type 2 diabetes. Because of its elevated prevalence, the contribution of type 2 diabetes as a major risk factor for cardiovascular disease is receiving considerable attention from the medical community. It has even been proposed that type 2 diabetes should be considered as an equivalent of coronary heart disease (CHD). As many complications of type 2 diabetes are related to hyperglycemia, achieving a better glycemic control is obviously a very important therapeutic objective. However, there is increasing evidence that hyperglycemia is not the main factor responsible for the increased CHD risk in type 2 diabetic patients. Rather, a cluster of metabolic complications (often referred to as the metabolic syndrome) including an atherogenic dyslipidemia, insulin resistance, a thrombotic and inflammatory profile, as well as endothelial dysfunction could substantially increase the risk of CHD. As the metabolic syndrome is largely a consequence of our "toxic" lifestyle, it has been proposed that abdominal obesity, especially visceral obesity, is the main form of the metabolic syndrome in our population. There is also evidence that the increased CHD risk related to the presence of the metabolic syndrome could be partly explained by metabolic abnormalities that are not currently assessed in daily clinical practice. It is therefore suggested that in order to optimally manage CHD risk in type 2 diabetic patients, attention should be given not only to the achievement of a better glycemic control, but also to the improvement of features of the metabolic syndrome. Recent results from clinical trials have shown that fibrate therapy can reduce the risk of CHD events in patients with the metabolic syndrome. Such benefits being partly independent from changes in HDL-cholesterol. As fenofibrate has been shown to improve several features of the atherogenic dyslipidemia of the metabolic syndrome, it is proposed that this drug could help manage high-risk low HDL-cholesterol patients with the metabolic syndrome. For the time being, we do not know which features of the metabolic syndrome (insulin resistance/hyperinsulinemia, small LDL particles, reduced adiponectin levels, increased CRP, etc.) are critical therapeutic targets for the optimal management of CHD risk in type 2 diabetic patients. Several candidates have been proposed and further work is clearly warranted to justify the use of these new risk markers in clinical practice.

DO WE UNDERSTAND THE MECHANISMS OF BENEFIT OF STATIN THERAPY?

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Landmark studies established that hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (or statins) lower total and low-density lipoprotein (LDL) cholesterol and decrease the risk of cardiovascular events, in patients with a past history of myocardial infarction. In addition, statin treatment administered immediately following an acute coronary event may also reduce the risk of recurrent cardiovascular events. Clinical and experimental evidence indicate that the benefits of statin therapy result from altered plaque biology rather than mere regression of stenoses. Inflammation contributes importantly to the destabilization of plaques that underlies most acute coronary syndromes. The benefits of statin treatment may result from improved plaque stability related to the anti-inflammatory properties of statins, as reflected in reduced C-reactive protein (CRP) levels. In addition, lipid lowering and direct effects of statins can augment local levels of nitric oxide, an endogenous vasodilator and anti-platelet mediator. Statin treatment may also promote a favorable balance between endogenous procoagulants and fibrinolytic mechanisms, and have a selective immunosuppressive effect mediated by reduced histocompatibility antigen expression. Inhibition of HMG-CoA reductase not lowers cholesterol levels but interferes with the prenylation of small G proteins involved in biological control and lowers levels of caveolin, a protein that reduces NO synthase activity. The beneficial effects of statin treatment on clinical cardiovascular outcomes thus probably arise from a combination of LDL-lowering and such lipoprotein-independent effects.

A NEW LOOK AT CARDIOVASCULAR DISEASE: STOPPING THE PROGRESSION OF ATHEROSCLEROSIS WITH AGGRESSIVE LIPID-LOWERING

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Over the last decade, a number of important randomized clinical trials have demonstrated that treatment with statin drugs reduce the cardiovascular morbidity and mortality in patients with known coronary disease as well as in those who are at risk of developing clinical coronary artery disease. However, optimal lipid management with statins and the impact of different agents on the clinical outcomes and on the progression of atherosclerosis are not known.

Intravascular ultrasound is a unique imaging modality that provides direct tomographic imaging of the vessel wall in the beating human heart. This imaging modality unlike angiography allows direct visualization of the atheroma. Serial intravascular ultrasound imaging is optimally suited to observe and quantify the changes that occur overtime in response to various interventions.

Recently, several serial intravascular ultrasound studies have been published. In the RAD trial, Everolimus was shown to be more efficacious than the standard treatment in reducing the severity and incidence of allograft vasculopathy determined by IVUS done 4 weeks and 1 year after cardiac transplantation. In a six-week serial study IVUS imaging showed HDL mimetic ApoA-1 Milano/phospholipid complex produced significant regression of atherosclerosis. In the REVERSAL trial, impact of aggressive lipid lowering compared to moderate lipid lowering on the progression of atherosclerosis was assessed using intravascular ultrasound. The primary endpoint (percent change in atheroma volume) showed a significantly lower progression rate in Atorvastatin (intensive) group (p=0.0.0.02). In the almost simultaneously published PROVE-IT trial investigators used the identical randomization in patients with acute coronary syndromes and conclusively demonstrated the superiority of the intensive lipid lowering therapy.

Coronary atherosclerosis is a very dynamic disease process. Its progression can be stopped and even regressed with aggressive lipid management. Even small changes in the atheroma volume can be quantified by using volumetric serial IVUS imaging.

EXPANDING THE SCOPE OF CLINICAL BENEFITS ACROSS DIFFERENT RISK COHORTS: DIABETIC PATIENTS

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Patients with diabetes are at increased risk for developing complications related to atherosclerosis. In addition to the 2- to 4-fold increased risk of cardiovascular disease (CVD), type 2 diabetes mellitus is associated with a higher case fatality postmyocardial infarction (MI) and a poorer outcome after interventional procedures. People with diabetes mellitus also experience high rates of out-of-hospital mortality, indicating the need for aggressive primary prevention.

In trials of primary and secondary prevention of coronary heart disease (CHD) with statins, risk reductions in CHD events were similar in patients with diabetes and in the overall patient population. In particular, the Heart Protection Study has shown that statin therapy should now be considered routinely for all diabetic patients at sufficiently high risk of major vascular events, irrespective of their initial cholesterol concentrations.

The GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study has assessed the effect of atorvastatin titrated from 10 to 80 mg/d versus "usual" medical care on morbidity and mortality (total and coronary) in 1600 patients with established CHD. During this study significantly more CHD patients on "usual" care (n = 196 [24.5%]) had a CHD recurrent event or died than with atorvastatin (n = 96 [12%]; risk ratio, 0.49; $P < 0.0001$). Moreover, all subgroups of patients (including women, those with diabetes mellitus, arterial hypertension, aged 60 to 75 years, congestive heart failure, recent unstable angina or prior revascularization) benefited from treatment with atorvastatin.

The effects of atorvastatin in the primary prevention of CVD in patients with type 2 diabetes mellitus are also currently being evaluated in the Collaborative AtoRvastatin Diabetes Study (CARDS) in which 2838 patients with type 2 diabetes mellitus with no previous MI or CHD were randomized to receive either atorvastatin 10 mg/d or placebo. CARDS was originally scheduled to be completed in 2005; however, the Data Safety Monitoring Board decided to terminate the study early due to a significant reduction in the incidence of CV events following treatment with atorvastatin.

In summary, these studies suggest that aggressive treatment of elevated lipid levels is key to the successful management of atherosclerosis in patients with diabetes mellitus.

EXPANDING THE SCOPE OF CLINICAL BENEFITS ACROSS DIFFERENT RISK COHORTS: HYPERTENSIVE PATIENTS

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ALLHAT and ASCOT-LLA evaluated the benefits of statins among hypertensive patients. Prior to this, randomized controlled trial data were available from analyses of the hypertensive subgroups in lipid-lowering trials in secondary and primary prevention and from the largest statin trial, the Heart Protection Study (HPS). In the HPS 41% of patients were hypertensive (only 1% however did not have a history of a cardiovascular event, active vascular disease, or diabetes). In PROSPER 62% of elderly patients were hypertensive. Like HPS, PROSPER mainly included patients with established vascular disease. Analyses show the benefits of lipid lowering – primarily with statins - are similar for hypertensive and normotensive patients. More surprising is that stroke risk was reduced by an average of 15 and 30% in primary and secondary prevention settings, respectively. ALLHAT compared the impact of 40 mg/day pravastatin with usual care in over 10,000 hypertensives. The differential effect of pravastatin on total and LDL cholesterol (11 and 17% respectively) was smaller than expected due to extensive statin use in the usual care group and was associated with a non-significant 9% reduction in fatal coronary heart disease and non-fatal myocardial infarction, and 9% reduction in fatal and non-fatal stroke. However ASCOT–LLA, which also included over 10 000 hypertensives, showed a 36% reduction in the primary endpoint of total coronary heart disease and non-fatal myocardial infarction and 27% reduction in fatal and non-fatal stroke associated with atorvastatin 10 mg/day compared with placebo in patients with total cholesterol ≤ 6.5 mmol/l. The difference seen in ALLHAT and ASCOT-LLA probably reflects the greater relative difference in total and LDL–cholesterol (24% and 35% respectively) among the actively treated groups in ASCOT. In view of the results of ASCOT and other trial data it seems reasonable to treat all patients <80 years with a total cholesterol >3.5 mmol/l (135mg/dl) who have an estimated 10-year cardiovascular risk of 20% or more with a statin. Given these lower thresholds for intervention it is inevitable that previously-recommended targets for therapy (total cholesterol <5mmol/l or LDL <3mmol/l or 25%/30% reduction respectively, whichever is the greater) should also fall. Hence '4 and 2' should replace '5 and 3' as optimal targets.

STATINS AND BROADENING THE SCOPE OF CARDIOVASCULAR BENEFITS: LDL LOWERING AND BEYOND

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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 such as the Heart Protection Study of simvastatin and the ASCOT of atorvastatin have renewed attention to the concept of basing treatment decisions on overall risk for developing coronary disease, rather than on the presence of any single risk factor. Furthermore, new data from the REVERSAL and PROVE-IT, trials have suggested the benefits of more aggressive cholesterol reduction compared with a moderate strategy. Taken as a whole, the trials have broadened substantially the kinds of patients who might be considered for statin treatment. A consequence of this shift may be increased demands on limited healthcare resources in many countries that will require a solution that will reconcile evidence-based medicine with financial realities. Better targeting of treatment by improving risk assessment techniques may help ease the burden.

THE IMPORTANCE OF CHOLESTEROL AND RELATED STEROLS IN LIPID MANAGEMENT

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The role of cholesterol and particularly low density lipoprotein (LDL) cholesterol as risk factor for atherosclerosis and associated diseases such as coronary heart disease is well accepted. Recent clinical trials comparing intensive lipid-lowering with moderate lipid-lowering therapies indicate that future treatment goals might be lower than current guidelines demand. But even with current guidelines many patients do not reach these goals, requiring additional lipid-lowering concepts. With the discovery of ezetimibe, inhibition of cholesterol absorption became a second standard concept in lipid-lowering therapy. Ezetimibe inhibits the absorption of cholesterol and related sterols such as plant sterols in the human intestine, resulting in a reduction in plasma LDL cholesterol in the range of 18-22%. Ezetimibe also inhibits the absorption of plant sterols such as sitosterol and campesterol. While their role as potential risk factor is yet unclear in normal subjects, in patients with homozygous sitosterolemia elevated plasma plant sterols levels are responsible for severe atherosclerotic lesions. This leads to a clinical impression similar to that observed in patients with familial hypercholesterolemia. In patients with homozygous sitosterolemia, ezetimibe lowers elevated plasma plant sterol levels, associated with a reduction in xanthoma volume. Like other agents that inhibit intestinal cholesterol absorption, ezetimibe also increases endogenous cholesterol synthesis. This results in an enhanced fecal excretion of biliary cholesterol which underscores the value of a combined therapy with statins. Several multi-centre trials have shown synergistic effects of the dual inhibition of cholesterol absorption and cholesterol synthesis, leading to a strong LDL cholesterol reduction even with low doses of statins, which allows more patients to reach current treatment goals compared to statin monotherapy.

FURTHER INSIGHTS ON THE EZETIMIBE MECHANISM OF ACTION, ROLE OF NPC1L1

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Ezetimibe (Zetia™, Ezetrol™), a cholesterol absorption inhibitor, has provided a new approach for reduction of elevated plasma cholesterol levels. Elucidation of the molecular mechanism of ezetimibe has been achieved with the identification of Niemann-Pick C1 Like 1 (NPC1L1), a protein localized in jejunal enterocytes that is critical for intestinal cholesterol absorption. The uptake of intestinal phytosterols and cholesterol into enterocytes was not fully defined on a molecular level, and the role of NPC1L1 in maintaining whole body cholesterol homeostasis is not known. While otherwise phenotypically normal, NPC1L1 (-/-) null mice have a 70% or greater decrease in absorbed cholesterol, which is unaffected by treatment with ezetimibe. NPC1L1 null mice also have a substantially reduced intestinal uptake of cholesterol and sitosterol, with dramatically reduced plasma phytosterol levels. NPC1L1 null mice are completely resistant to diet-induced hypercholesterolemia, with lipoprotein and hepatic cholesterol profiles similar to those of wild type mice treated with ezetimibe. Cholesterol feeding results in down regulation of intestinal NPC1L1 mRNA expression in wild type mice. NPC1L1 deficiency results in an increase in intestinal cholesterol synthesis, down regulation of ABCA1 mRNA, and no change in ABCG5 and ABCG8 mRNA expression. NPC1L1 is required for intestinal uptake of both cholesterol and phytosterols, and plays a major role in cholesterol homeostasis. Ezetimibe has no effect in NPC1L1 knock-out mice, indicating that NPC1L1 resides in the ezetimibe-sensitive pathway responsible for the majority of intestinal cholesterol and phytosterol absorption. Thus, NPC1L1 may be a useful drug target for the treatment of hypercholesterolemia and sitosterolemia.

EZETIMIBE AND EZETIMIBE/SIMVASTATIN CLINICAL UPDATE

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It is well established that many patients displaying hypercholesterolemia do not reach the adequate goal upon current lipid lowering therapy. Furthermore, recent evidence stressed the importance of aggressive therapy in patients at high risk for cardiovascular disease. For these reasons there is increasing interest in the combined therapy notably in patients with severe hypercholesterolemia or patients at high risk for cardiovascular disease. Ezetimibe is the first compound of a new class of selective cholesterol absorption inhibitors. The drug impairs the intestinal absorption of both intestinal and dietary cholesterol through inhibition of the recently identified cholesterol receptor (Niemann-Pick C1 like 1 : NPCILI). Since statin therapy is almost mandatory in patients with LDL-c above the threshold level determined depending on the cardiovascular risk, ezetimibe has been extensively studied in combination with statins. In association with these drugs, ezetimibe further decreases LDL-c by 18-20%. The increase in HDL-c and the decrease of triglyceride levels are more modest and not consistent across different trials. The drug was given to a variety of populations and was shown to have similar efficacy in all populations studied so far. In collaboration with others we have studied the effect of ezetimibe in patients suffering homozygous hypercholesterolemia (Circulation 2002, 105(21) :2469-75), a condition in which current pharmacological treatment have limited efficacy. In this population, ezetimibe decreased LDL-c by 20%, a result which is similar to the efficacy of the highest dosage of simvastatin or atorvastatin. In addition to the decrease of LDL-c, ezetimibe was shown to lower phytosterol levels in patients with the rare disorder called sitosterolemia, a condition leading to premature atherosclerosis. This result is explained by the role of the NPCILI in the absorption of both cholesterol and phytosterols.
Globally, ezetimibe was found to be safe and the incidence of side effect similar to placebo. Due to the absence of metabolism by the cytochrome P 450 there are few interactions to be expected. This is noticeable in patients at high risk already treated with many drugs.
In conclusion, ezetimibe is a safe drug acting through a novel mechanism of action and offers new perspectives in the treatment of hypercholesterolemic patients.

OUTCOMES STUDY PROGRAM UPDATE

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Aggressive lowering of LDL-cholesterol (LDL-C) has been shown to effectively reduce atherosclerosis and cardiovascular events in a broad array of patients, but there are still conditions and patient populations where such therapy has been poorly documented. Ezetimibe in co-administration with a statin has emerged as an attractive combination therapy to reduce LDL-C effectively, and an active cardiovascular clinical study program is ongoing.

The Study of Heart and Renal Protection (SHARP) aims at demonstrating the efficacy on reducing major vascular events of such combination therapy in patients with chronic kidney disease (creatinine 130μmol/L in women or 150μmol/L in men) or receiving dialysis. The study will include 9000 patients across the world to be randomized to ezetimibe 10 mg + simvastatin 20 mg or placebo, to be followed for about 4 years.

The Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) study will compare the effect on change in carotid artery intima media thickness of ezetimibe 10 mg + simvastatin 80 mg with simvastatin 80 mg alone.

LDL-C has been identified as an important risk factors for the degenerative type of aortic valve stenosis along with age, male sex, triglycerides and low HDL-cholesterol as well as diabetes, hypertension and smoking. An "early lesion" of the aortic valve leaflets has a lot in common with the early lesion in atherosclerotic plaques. We therefore designed a clinical trial with the main objective to determine whether aggressive lowering of LDL-C with the combination of simvastatin 40 mg and ezetimibe 10 mg daily will slow down the progression of disease in patients with asymptomatic aortic valve stenosis and without signs or symptoms of atherosclerotic disease. The Simvastatin + Ezetimibe in Aortic Stenosis (SEAS) study is an international multicentre, randomized, placebo-controlled double-blind clinical study in 1873 patients with moderate aortic valve stenosis. The main endpoint is cardiac death or aortic valve replacement or major cardiovascular events. The patients are followed with annual echocardiographic examinations. Results are expected in 2007.

THE LONG PENTRAXIN PTX3: FROM INNATE IMMUNITY TO HISCHEMIC HEART DISORDERS.

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The long pentraxin PTX3 was originally identified (cDNA and genomic, mouse and human) as an IL-1 inducible gene in endothelial cells. PTX3 is a prototypic long pentraxin consisting of a C terminal pentraxin-like domain with similarity to C Reactive Protein (CRP) and Serum Amiloid P Component (SAP), coupled to an unrelated N terminal portion. Unlike CRP, produced in the liver, it is induced by inflammatory signals mainly in endothelial cells and macrophages. Recent data in gene targeted mice show that PTX3 is a unique soluble pattern recognition receptor playing a non redundant role in innate immunity and female fertility. PTX3 is produced in atherosclerotic lesions and levels increase in hischemic heart disorders. In order to assess the prognostic value of PTX3 in myocardial infarction we have analyzed a large group of patients comparing the prognostic value of PTX3 with other cardiac biomarkers, such as troponin T and N-terminal pro-brain natriuretic peptide, or with the liver-derived short pentraxin CRP. Multivariate logistic analysis was used to analyze the data. The results on the prognostic value of PTX3 in acute myocardial infarction will be discussed.

EFFECT OF THE ANTIOXYDANT FEAOX6 AND FEAOX335 ON FOAM CELL FORMATION AND INFLAMMATORY RESPONSE

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A pivotal role in atherogenesis is played by macrophages, which are early site for lipid accumulation and mediate the inflammatory and immune response in the intima. Mixtures of tocotrienols are proposed to be beneficial both to prevent and slow atherosclerosis. However, little is know on the effects of single tocotrienols on macrophage atherogenic role. This study evaluated the effects on foam cell formation and inflammatory response of treatment with the alpha-tocotrienol (FEAOX335) and the antioxidant FEAOX6, recently obtained through molecular combination by us [1]. [^{3}H]oleate incorporation into cholesteryl ester in the presence of either native (nLDL), acetylated (acLDL), oxidised (oxLDL) LDL or in their absence was evaluated in HMDM (Human Monocyte Derived Macrophage) treated (24h) with 0 (untreated), 5 and 20 μM of FEAOX6 or FEAOX335. These compounds inhibit, in a dose-dependent manner, the synthesis of [^{3}H]cholesteryl ester. The reduction is significant with acLDL in cells treated with 20μM of FEAOX6 and FEAOX335 (78±33 and 57±19 pmol/h/mg protein, respectively) in comparison with untreated cells (193±58 pmol/h/mg protein). FEAOX335 also decreases cholesterol esterification induced by oxLDL (51±15 and 25±6 pmol/h/mg protein in FEAOX335–treated and untreated, respectively). The inflammatory response induced by LPS and evaluated by arachidonic acid release, is lower in FEAOX335- than in FEAOX6- treated cells. However, at concentration of 20 μM, but not 5μM, the study compounds raise arachidonic acid release by macrophages. These results strongly support a dose-dependent beneficial role of tocotrienols on macrophage functions, and suggest that FEAOX335 has better potentialities than FEAOX6.

[1] Palozza et al.FRBM 2002;33:1724-3.

CIRCULATING OXIDISED LDL AND ISOPROSTANES ARE ASSOCIATED WITH DIFFERENT PARAMETERS OF THE METABOLIC SYNDROME.

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The metabolic syndrome predisposes to the development of cardiovascular diseases, explained by an accumulation of atherogenic risk factors, such as insulin resistance, dyslipidemia and abdominal obesity. Recently, elevated circulating oxidized LDL (oxLDL) concentrations, and oxidative stress have been proposed as additional features of the metabolic syndrome. Therefore, we investigated relationships between circulating oxLDL, urine isoprostanes (as a marker of oxidative stress) and parameters of the metabolic syndrome in 309 healthy men (all 63 years). Fasting insulin, lipid and lipoprotein concentrations, and anthropometric measurements were available. Circulating oxLDL concentrations were determined with the monoclonal antibody Mab-4E6 (ELISA) in EDTA-plasma samples. Isoprostanes were measured in 24-hour urine samples. Isoprostanes and oxLDL were not correlated to each other. Furthermore, oxLDL but not isoprostanes, were positively correlated with typical dyslipidaemic parameters (such as plasma triglycerides, total cholesterol, LDL cholesterol, small dense LDL and apoB (all $p<0.0001$)). In turn, isoprostanes but not oxLDL showed positive relationships to insulin resistance (fasting plasma insulin, proinsulin and HOMA-index (all $p<0.05$)) and to the different anthropometric measurements (BMI, WHR and sagittal abdominal diameter (all $p<0.05$)). These results indicate that both oxLDL and oxidative stress (isoprostanes) are associated with parameters of the metabolic syndrome. However, the divergence in correlations suggest oxLDL and isoprostanes to reflect different processes. To extend these studies we will quantify autoantibodies against modified LDL, markers of inflammation and endothelial activation and relate these parameters to oxidative stress and circulating oxLDL.

PAF-AH ACTIVITY INDICATES ANGIOGRAPHIC CAD RISK INDEPENDENT OF SYSTEMIC INFLAMMATION AND OTHER RISK FACTORS

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Background: Platelet activating factor acetyl hydrolase (PAF-AH), also denoted as lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme involved in inflammation and associated with the atherogenic lipoprotein phenotype and increased risk of CAD. This study defines the significance of PAF-AH activity (PAF-AH-act) to angiographic CAD and its relationship to other established risk factors.

Methods and Results: PAF-AH-act, lipoproteins, sensitive CRP (sCRP), fibrinogen, serum amyloid A (SAA) and white blood cell count (WBC) were determined in 2454 subjects with angiographically confirmed CAD and in 694 control subjects. PAF-AH-act was highly correlated with LDL cholesterol (LDL-C) (r=0.517), apolipoprotein B (r=0.644) and non-HDL cholesterol (nHDL-C) (r=0.648), but not with sCRP or fibrinogen. PAF-AH-act was significantly lower in women than in men and significantly lower in individuals using lipid-lowering drugs (LLDs) compared to non-users. Unlike sCRP, fibrinogen, and SAA, PAF-AH-act was not elevated in acute coronary syndromes. After adjusting for the use of, or when examining only non-users of LLDs, PAF-AH-act quartiles were significantly associated with CAD (OR 1.24, 95 % CI 1.15 –1.35, $p<0.001$, and OR 1.31, 95 % CI 1.19 – 1.43, $p<0.001$, respectively). This association was independent of established cardiovascular risk factors; it was attenuated, but not abolished by lipoprotein parameters like LDL-C.

Conclusions: PAF-AH-act is not an indicator of systemic inflammation accompanying atherosclerosis. However, PAF-AH-act indicates elevated risk of CAD complementary to sCRP and independent of established risk factors like LDL-C.

MODIFIED LIPOPROTEINS AND ENDOTHELIAL DYSFUNCTION

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The molecular mechanisms underlying the relationship between elevated plasma concentration of triglyceride-rich lipoproteins and coronary artery disease remain uncertain, evidence is accumulating to suggest that endothelial dysfunction is involved. In the present work we addressed two major questions: first we investigated the gene expression pattern and intracellular pathways in human endothelial cells incubated with very low density lipoproteins (VLDL) and oxidatively modified VLDL (Ox-VLDL). Second we investigated verify whether changes in RLPs plasma levels during the postprandial phase relate to alterations of the endothelial function

Among 8411 genes spotted on the array, 1620 (19,2%) were expressed under basal condition. VLDL predominantly activated the ERK1/2 pathway while P38 MAPK was the main target of Ox-VLDL, and CREB and NF-KB were activated by both VLDL and Ox-VLDL. VLDL induced MMP-2 (5.47 ±1.74 fold), CD38 (2.38 ±0.23) and TGF alpha (2.51 ±0.3) expression. Ox-VLDL was found to induce IL-15 (2.10 ±0.48) and MIF (3.19 ±0.07) expression, to promote the generation of reactive oxygen species and to exert a cytotoxic effect, thus sustaining inflammation and endothelial damage. These findings confirm the involvement of VLDL and Ox-VLDL in endothelial dysfunction and suggest new genes and molecular mechanisms involved in these actions. Moreover cholesterol in RLPs contribute significantly to the endothelial dysfunction occurring during the postprandial lipemia.

HDL SUBFRACTION METABOLISM AND ATHEROSCLEROSIS: WHAT THE ZEBRA CAN TELL US ABOUT THE HORSE

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The first step in HDL metabolism is the synthesis of apolipoprotein (apo) A-I. When this does not occur there is virtually no HDL in plasma and the patients develop premature CHD (as in apoAI/CIII/AIV or apoAI/CIII or apoAI deficiency states). The second step is the efflux of free cholesterol from cells via ATP binding cassette protein A1 (ABCA1). When this does not occur there is only very small discoidal HDL in plasma that has pre-beta 1 mobility, and is rapidly cleared by the kidney. These patients develop premature CHD. Their LDL is small, triglyceride and beta carotene enriched, and is also hypercatabolized (Tangier disease). Pre-beta 1 HDL serves as the best acceptor of free cholesterol via ABCA1 from macrophages and other cells, although apoA-I Milano-phospholipid complexes may be even better. The third step is cholesterol esterification via lecithin cholesterylacyltransferase (LCAT). When this does not occur there is pre-beta 1 HDL and small discoidal HDL of alpha mobility (alpha 4 HDL, which is hypercatabolized. These patients have corneal opacification, and can develop kidney disease and hypersplenism. The fourth step is remodeling of HDL via: a. lipoprotein lipase, b. endothelial lipase, c. phospholipid transfer protein, d) cholesteryl ester transfer protein (CETP), and e. hepatic lipase deficiency. In lipoprotein lipase deficiency there is hypertriglyceridemia, and small dense TG rich HDL that is hypercatabolized, and in the heterozygous state premature CHD. In contrast with CETP deficiency very large abnormal HDL are formed which contain both apoA-I and apoA-II. These particles may not be as effective in delivering cholesteryl esters to the liver via scavenger receptor B1 (SRB1) (the fifth step in HDL metabolism). It does appear that the catabolism of apoA-I is slower than that of cholesteryl esters on HDL, and that after removal of CE by the liver small alpha 4 particles are formed, which can then go through the HDL cycle again. Patients with premature CHD tend to have overproduction of TG rich particles, decreased LDL clearance, and small dense LDL and small dense HDL (decreased alpha 1 HDL and increased pre-beta 1 HDL). Alpha 1 HDL serves as the particle that interacts best with the SRB1 receptor. CHD patients are also more likely to have increased CETP activity and decreased LPL activity. Our data do suggest that CETP inhibition by about 50% should decrease CHD risk significantly (based on our studies in Framingham and VA-HIT). This concept is currently being tested in clinical trials.

INSULIN RESISTANCE AND DYSLIPIDEMIA: THE ROLE OF PPAR GAMMA

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Increased assembly and secretion of very low density lipoproteins (VLDL) by the liver is a central abnormality in the hypertriglyceridemia (HiTG) of insulin resistance/diabetes. The causes of increased VLDL secretion are multiple. Studies in cultured liver cells, and in animal models and humans, suggest availability of fatty acids (FA) for synthesis of TG and cholesteryl esters (CE) is a key determinant of whether newly synthesized apolipoprotein B (apoB) is degraded or secreted as a lipoprotein. The sources of FA can be from the uptake of plasma albumin-bound FA, FA delivered as TG and CE in lipoprotein remnants that are taken up by endocytosis, and FA synthesis as part of hepatic de novo lipogenesis (DNL). Evidence in animal models and humans support a major role for increased plasma FA flux as a stimulus for VLDL secretion. Similar studies indicate that remnant uptake is also a stimulus for VLDL secretion. Less is known about lipogenesis: in mice, it is clearly important but the data in humans is less certain. SREBP-1c seems important in some, but not all mouse models of increased VLDL secretion. However, hepatic PPAR gamma gene expression is also increased in many of those models. Studies of overexpression or targeted disruption of PPAR gamma in the liver support its importance in VLDL secretion. In the apoB/BATless mouse, a model of insulin resistance, obesity and hyperlipidemia, SREBP-1c mRNA and protein are not increase while PPAR gamma2 gene expression is elevated, as are mRNA levels of several PPAR gamma2 target genes and genes important for lipogenesis. Studies are underway to determine if PPAR gamma2 is directly linked to increased lipogenesis in the apoB/BATless mouse.

THE FATTY LIVER: A KEY TO INSULIN RESISTANCE IN BOTH OBESITY AND LIPOATROPHY

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NAFLD encompasses a spectrum of liver pathology ranging from non-alcoholic steatosis to steatohepatitis. In the NHANES III survey, the prevalence of elevated liver enzyme values was 7.9%, of which 69% was attributed to NAFLD (6.4 million adults in the US). NAFLD was strongly associated with features of insulin resistance *(Am J Gastroenterol 98:960)*. NAFLD increases the risk of type 2 diabetes independent of obesity, and cirrhosis following NAFLD is the 3rd leading cause for liver transplantation. The causes of variation of liver fat content independent of obesity are poorly understood. A fatty liver is not just commonly found in obese subjects but also in patients with lipoatrophy *(AIDS 16:2183)*. Intra-andominal does not explain all of the variation in liver fat. Regarding additional factors, adiponectin deficiency characterizes both lipodystrophic and obese insulin resistant individuals, and serum levels correlate with liver fat content *(JCEM 88:1907, 89:200)*. Treatment of both lipodystrophic and type 2 diabetic patients with PPARγ agonists but not metformin decreases liver fat and increases adiponectin *(Antivir Ther 8:199, JCEM 89:200, Diabetes in press)*.

Conclusions: Fat accumulation in the liver, which is not due to excessive alcohol consumption or known other cause, is a proximal correlate of insulin resistance in both men and women, independent of overall obesity. PPARγ agonists unlike other antidiabetic agents such as metfromin are able to decrease liver fat.

SOLUBLE CELLULAR ADHESION MOLECULES IN PATIENTS WITH DIABETES MELLITUS: RELATION TO DIABETIC MICROVASCULAR COMPLICATIONS

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Cellular adhesion molecules (CAMs) may be involved in the development of diabetic complications. The aim of the present study was to investigate the relation of serum CAMs to diabetic microvascular complications (nephropathy, retinopathy and peripheral neuropathy) in patients with diabetes mellitus (DM).

Forty-three patients with type 1 DM and 38 patients with type 2 DM had fasting serum levels of soluble ICAM-1, VCAM-1 and P-selectin determined. The albumine/creatinine ratio was measured in the first morning urine and a ratio > 2 g/mol was indicative of microalbuminuria (and nephropathy). The vibration threshold at the medial malleol was measured to diagnose peripheral neuropathy, while the presence or absence of diabetic retinopathy was determined by an ophthalmologist.

Patients with microalbuminuria had higher mean P-selectin than patients with normoalbuminuria (109±36 vs.84±28, $p<0.05$) with a non-significant trend toward higher ICAM-1 (315±73 vs. 288±69) and VCAM-1 (918±246 vs. 780±246). Divided in tertiles, patients with the highest vibration threshold had higher mean P-selectin (90±24 vs. 76±22, $p<0.05$) and a non-significant trend towards higher ICAM-1 (305±71 vs. 274±65) and VCAM-1 (862±310 vs. 743±192). A trend towards higher mean P-selectin (94±29 vs. 84±30) and VCAM-1 (872±244 vs. 766±245) in patients with retinopathy was not significant. In patients with type 1 DM, P-selectin correlated with HbA1C ($r=0.434$, $p<0.01$). Patients with type 2 DM had a close correlation between body mass index and VCAM-1 ($r=0.466$, $p<0.01$).

Microalbuminuria and peripheral neuropathy were associated with higher serum levels of soluble P-selectin in diabetic patients. Future studies should evaluate a possible causal role of CAMs in the development of diabetic microvascular complications.

SUPPRESSION OF RAGE AS A BASIS OF SIMVASTATIN-DEPENDENT PLAQUE STABILIZATION IN TYPE 2 DIABETES.

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The clinical benefits of statins in diabetes are attributed to changes in plaque composition leading to reduced metalloproteinase (MMP) activity and plaque stabilization. However, the molecular mechanism(s) underlying this effect are not completely elucidated. Strong evidences suggest a central role for RAGE (receptor for advanced glycation end products) in the accelerated progression of atherosclerosis observed in diabetes. In particular, we have recently demonstrated increased expression of RAGE in human diabetic plaques, in association with inducible cyclooxygenase and PGE synthase-1 (COX-2/mPGES-1) overexpression, and PGE_2-dependent MMP generation leading to plaque rupture. Thus, the aim of this study was to characterize the effect of simvastatin on the inflammatory infiltration and the expression of RAGE and RAGE-dependent plaque-destabilizing genes in human carotid plaques. Seventy type 2 diabetic patients with asymptomatic carotid artery stenosis (>70%) were randomized to American Heart Association step 1 diet plus simvastatin (40 mg/d) or American Heart Association step 1 diet alone for 4 months before endarterectomy. Plaques were subjected to analysis of RAGE, COX-2, mPGES-1, MMP-2 and MMP-9, lipid and oxidized LDL (oxLDL) content, and collagen content by immunocytochemistry, Western blot and RT-PCR, whereas zymography was used to detect MMP activity. Immunocytochemistry was also used to identify CD68+ macrophages, CD3+ T-lymphocytes, smooth muscle cells (SMCs) and HLA-DR+ inflammatory cells. Plaques from simvastatin group had fewer ($P<0.0001$) macrophages, T-lymphocytes, and HLA-DR+ cells; less ($P<0.0001$) immunoreactivity for RAGE (9±2% vs 25±5%), COX-2 (11±3% vs 26±4%), mPGES-1 (5±1% vs 22±4%), MMP-2 (8±3% vs 24±4%), and MMP-9 (10±3% vs 26±6%); reduced ($P<0.0001$) gelatinolytic activity; increased ($P<0.0001$) collagen content, and reduced ($P<0.0001$) lipid and oxLDL content. Interestingly, RAGE inhibition by simvastatin was observed not only in plaque sections but also in plaque-derived macrophages. In conclusion, this study demonstrates that simvastatin decreases inflammation and inhibits RAGE expression in plaque macrophages, and this effect in turn may contribute to human plaque stabilization by inhibition of PGE_2-dependent biosynthesis of metalloproteinases responsible for plaque rupture.

PREATHEROSCLEROSIS AND ADIPOCYTOKINES IN JUVENILE OBESITY

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Background: Adipocytokines are centrally involved in metabolic abnormalities of obesity. To investigate their involvement in pre-atherosclerosis, we measured serum levels of resistin, leptin, leptin receptor, and adiponectin in obese juveniles that were previously found with increased intima media thickness (IMT) of common carotid arteries (CCA) and increased low grade inflammation, and compared them to healthy, normal weighted, juvenile controls.

Methods: Serum/plasma levels of ultra sensitive C-reactive protein (US-CRP), malondialdehyd (MDA), lipid fractions, glucose, homocysteine, resistin, leptin, leptin receptor, and adiponectin were determined by means of ELISA in 199 obese juveniles (BMI-SDS: 6±1.6, age: 13±2.9 years, mean±SEM) and 203 normal wheigted age matched controls. Intima-media thickness (IMT) of both common carotid arteries (CCA) was measured by ultrasonography.

Results: Plasma levels of resistin were significantly positively correlated with US-CRP ($r=0.42$, $p<0.0001$) indicating the close link between inflammation and incipient type II diabetes in obesity. Leptin receptor was markedly decreased in the obese cohort ($p<0.01$) and correlated negatively with systolic blood pressure ($r = -0.3$, $p<0.01$) and IMT values ($r = -0.36$, $p<0.01$). Interestingly, adiponectin showed the best correlation with the increased IMT ($r = -0.46$, $p<0.0001$) emphasizing a protective function of adiponectin for the vessel wall. Obese probands with markedly decreased adiponectin showed increased MDA levels ($r=0.26$, $p<0.05$) indicating oxidative stress associated with low adiponectin levels. In line with this, HDL-cholesterol correlated positively with adiponectin ($r=0.33$, $p<0.002$).

Conclusion. Our data clearly indicate the close relationship between decreased adiponectin release, decreased HDL cholesterol, increased oxidative stress, incipient type II diabetes, hypertension, and upregulated inflammation in juvenile obesity. These metabolic abnormalities are definitely linked to preatherosclerotic symptoms (i.e. increased IMT). Thus, adiponectin shows a pronounced protective function for the vascular wall.

GLYCOCALYX DECREASE IN DIABETES MELLITUS AS NOVEL CAUSE FOR ATHEROSCLEROTIC VULNERABILITY

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Background: Diabetic patients show increased vulnerability for atherogenic insult, possibly related to increased vascular permeability. Recently, the pivotal role of the glycocalyx (the proteoglycan layer covering the endothelium) has been established as a determinant of the vascular permeability barrier. To assess whether glycocalyx perturbations may contribute to increased vascular permeability and atherogenic vulnerability in hyperglycemia, we studied the effects of acute and chronic hyperglycemia on whole body glycocalyx volume in type I diabetes mellitus subjects (DM-1) and healthy volunteers.

Methodology: Glycocalyx volume was determined by comparing the intravascular distribution volumes for Dextran 40 (glycocalyx permeable tracer) and autologous labeled erythrocytes (glycocalyx impermeable tracer). We then quantified glycocalyx volume in 6 young male DM-1 patients without diabetic complications and 7_matched control subjects (Con). In addition, glycocalyx measurements were performed in 7 healthy male volunteers after 6 hours normo-insulinemic hyperglycemia (HG: 14-16 mmol/l) using octreotide/glucose clamping. Measurements were repeated 24 hours later (NG: 3,8-5,6 mmol/l). In addition to glycocalyx volume endothelial function was assessed under both conditions.

Results: In DM-1 patients glycocalyx volume was diminished significantly compared to controls (DM-1: 0.9 ± 1.4 vs. Con: 2.9 ± 1.7 liters, $p < 0.05$) and accompanied by increased vascular permeability for Dextran 40. In volunteers, hyperglycemia resulted in a decrease of glycocalyx volume compared to controls (HG: 1.2 ± 1.7 liters, $p < 0.01$). After 24 hours glycocalyx volume partially recovered (NG: 2.0 ± 1.8 liters). Hyperglycemia induced glycocalyx perturbation was accompanied by FMD impairment (HG: 6.5 ± 0.9%, NG: 8.7 ± 0.8 %, $p< 0.01$).

Conclusion: In DM-1 patients vascular glycocalyx volume is severely diminished. In volunteers, similar reductions are seen within 6 hours of hyperglycemic clamping accompanied by the induction of endothelial dysfunction. These novel data imply that glycocalyx perturbation caused by hyperglycemia may be the missing link between increased vascular permeability and atherosclerotic vulnerability in patients with diabetes mellitus.

AN AYURVEDIC MEDICINE FOR DIABETES IMPROVES CARDIAC ABNORMALITY OF LIPID METABOLISM IN OBESE ZUCKER RATS: ROLE OF INHIBITING PPAR-α EXPRESSION IN THE HEART

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Cardiovascular disease is the major cause of morbidity and mortality in patients with diabetes. In hyperlipidemic states, accumulation of excess lipid in nonadipose tissues enlarges the intracellular pool of fatty acyl-CoA, thereby providing substrate for nonoxidative metabolic pathways that lead to cell dysfunction and death. Triglyceride accumulation in the heart is an important factor for development of diabetic cardiomyopathy. Therefore, improvement of abnormal cardiac lipid metabolism may prevent diabetes-related cardiac complications. Peroxisome proliferator-activated receptor alpha (PPAR-α) plays an important role in maintaining homeostasis of lipid metabolism, especially that of triglyceride[1]. Physiological triglyceride lipolysis mediated by lipoprotein lipase (LPL) occurs subsequent to PPAR-α activation and its distal transcriptional effects[1]. In contrast to circulating system [not sure what this means], PPAR-α activation by agonists could inhibit cardiac LPL as a protective mechanism against a potentially toxic oversupply of fatty acids to the myocardium[2]. *Salacia (S.) oblonga* (Celastraceae) root is an Ayurvedic medicine for diabetes and obesity. The objective of this study was to explore the effects of water extract isolated from *S. oblonga* on cardiac lipid metabolism and circulating lipid levels in obese Zucker rats (OZR). OZR and lean Zucker rats (LZR) were treated with the extract (100 mg/kg) or vehicle for 6 weeks. Enzymatic assays demonstrated that the extract markedly lowered increased triglyceride levels, but not total cholesterol levels, in both the heart and blood of OZR, whereas it did not affect these in LZR. Furthermore, semi-quantitative RT-PCR results showed that overexpression of PPAR-α and LPL mRNA in the OZR heart was strongly suppressed by the extract. Through a gene-reporter assay, it was demonstrated that the extract and one of its main components, mangiferin, concentration-dependently activated PPAR-α and LPL mRNA expression in macrophages. The activations were completely suppressed by a selective PPAR-α antagonist MK-886, further confirming the activities of the extract and the component and suggesting PPAR-α–dependent LPL activation. Our findings suggest that *S. oblonga* is an herbal PPAR-α activator and that its inhibition of enhanced PPAR-α in the heart may play an important role in improving cardiac lipid metabolism in diabetes.

TRANSPORT AND PROCESSING OF VLDL PARTICLES FROM LIVER TO OVARY

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Yolk is the major source of nutrients for the developing chicken embryo, but molecular details of the delivery mechanisms are largely unknown. During oogenesis in the chicken, the main yolk components vitellogenin and very low density lipoprotein (VLDL) are taken up into the oocytes via a member of the low density lipoprotein receptor gene family termed LR8 (*EMBO J.* 13, 5165-5175). This endocytosis is accompanied by partial degradation of the yolk precursors' protein moieties; however, fragmentation does not abolish binding of VLDL to LR8. The receptor exists in two isoforms that differ by a so-called O-linked sugar domain; the shorter form (LR8-) is the major form in oocytes, and the longer protein (LR8+) predominates in somatic cells. Both LR8 isoforms are expressed, at ratios that vary with embryonic age, in the extraembryonic yolk sac, which mobilizes yolk for utilization by the embryo, and in the allantois, the embryo's catabolic sink. Stored yolk VLDL interacts with LR8 localized on the surface of the yolk sac endodermal endothelial cells (EEC), is internalized, and degraded, as demonstrated by the catabolism of fluorescently labelled VLDL in cultured EEC. Addition to the incubation medium of RAP, which inhibits all known LR8/ligand interactions, blocks the uptake of VLDL by EEC. Importantly, EEC express significant levels of microsomal triglyceride transfer protein and protein disulfide isomerase, key components required for lipoprotein synthesis. The apolipoprotein pattern of VLDL isolated from the yolk sac-efferent vein is very different from that of yolk VLDL, strongly suggesting that embryo plasma VLDL is re-synthesized in the EEC. Thus, LR8 is a key mediator of a two-step pathway, which effects the uptake of VLDL from the yolk sac and the subsequent delivery of its components to the growing embryo.

PHYSIOLOGY AND CARDIOVASCULAR RISK OF APO B LIPOPROTEIN PARTICLE TYPES

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High triglycerides is an independent risk factor for coronary heart disease (CHD). Triglycerides are carried on a diverse group of VLDL and LDL, and these particles have distinct relationships to CHD, stronger or weaker. Small apolipoproteins, including apoCs, apoE and lipoprotein lipase, are present on a portion of TG-rich lipoproteins (TRL), and they modulate metabolism and perhaps atherogenicity. The concentration of VLDL and LDL particles that have apoCIII is a strong predictor of progression of atherosclerosis and independently predicts incidence of CHD even in statin-treated patients. These particles eclipse the association between TG and CHD. In type 2 diabetic patients, a high concentration of LDL particles that have apoCIII had a strikingly adverse relation to coronary events. In contrast, VLDL with apoCIII, a very big TG-rich particle, was not significantly predictive of coronary events, and in fact trended inversely, perhaps because these particles are too big to pass through the arterial endothelium. Kinetic studies in normal humans show that LDL with apoCIII are formed mostly from lipolysis of nascent VLDL with apoCIII, have relatively low concentration, e.g. 10 mg/dl, and may be considered remnant lipoproteins. Loss of apoCIII from VLDL during lipolysis is an important pathway for the formation of the major type plasma LDL that does not have apoCIII. The presence of apoE facilitates the clearance of apoCIII containing particles preventing them from being metabolized to LDL. Thus, favorable metabolism of apoB lipoproteins depends on formation by the liver of VLDL with apoE, as well as on removal of apoCIII during circulation presumably to HDL. Lipoprotein remants with apoCIII are cholesterol-rich, adhere to arterial proteoglycan, and stimulate adhesion of monocytes to endothelial cells. Thus, TRL remnants rich in apoCIII form an apoB lipoprotein system that is strongly linked to atherosclerosis and clinical CHD.

EFFECT OF SCAVENGER RECEPTOR B-I (SR-BI) DEFICIENCY ON HEPATIC TRIGLYCERIDE METABOLISM

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In addition to its role in the selective uptake of cholesterol esters from HDL, SR-BI is important in facilitating (hepatic) chylomicron remnant metabolism. In the current study we determined the effect of SR-BI deficiency on the expression of genes important in the regulation of intrahepatic cholesterol and triglyceride metabolism. Hepatic cholesterol levels were not changed between SR-BI wild-type (WT) and knockout (KO) mice were. In contrast, hepatic triglyceride levels in SR-BI KO mice were 60% decreased. The hepatic mRNA expression of CYP7A1 was 70% and CYP27 was 32% decreased in SR-BI KO mice vs WT mice. No significant change in the expression of ABCA1, ABCG5/G8, and BSEP was observed between SR-BI KO and WT mice. The expression of VLDL synthesizing genes FAS, DGAT and MTP was not different between SR-BI KO and WT mice, while PPARδ and HMGCS2 were respectively 27% and 54% downregulated in the SR-BI KO mice.
In conclusion, SR-BI deficiency does not affect hepatic cholesterol levels due to a compensatory decrease in the expression of bile acid synthesizing enzymes. In contrast, SR-BI deficiency leads to a markedly (60%) lower triglyceride level in the liver, establishing that the SR-BI mediated uptake of postprandial triglycerides is important for regulating liver triglyceride homeostasis.

PROBUCOL STABILIZES HEPATIC SCAVENGER RECEPTOR CLASS B TYPE I POSSIBLY THROUGH SPECIES-SPECIFIC FASHION

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Reverse cholesterol transport (RCT) is one of the major protective systems against atherosclerosis. In this system, HDL removes cholesterol from peripheral cells and delivers it to the liver, the terminal of RCT. Scavenger receptor class B type I (SR-BI) is a major receptor for HDL in the liver and the over-expression of SR-BI decreased atherosclerosis with concomitant reduction of HDL-cholesterol in murine models. On the other hand, probucol is known to be a potent hypolipidemic drug to regress xanthoma formation and carotid atherosclerosis, along with a marked reduction of HDL-cholesterol levels. The initial aim of the present study was to know whether or not probucol up-regulated SR-BI. First, we tested whether probucol increased SR-BI in the following *in vivo* and *in vitro* models from different species. Probucol up-regulated SR-BI in a dose-dependent fashion in human hepatoma cell line, HepG2, and primary hepatocytes. The uptake of HDL-lipids was increased in the probucol-treated HepG2 cells. Probucol increased rabbit SR-BI protein *in vivo*, using Japanese white rabbit as a model. However, this effect was not observed in wild type C57Bl6 mice. Using the HepG2 cells, we further investigated the mechanism for up-regulation of SR-BI protein. In the HepG2 cells, SR-BI mRNA was slightly increased. The most striking finding of this study was that the decay curve of SR-BI protein was markedly retarded in probucol-treated HepG2 cells in the presence of cycloheximide, indicating that probucol may stabilize human SR-BI protein. These findings were supported by the data showing that the addition of some protease inhibitors into the media increased the basal levels of SR-BI protein in HepG2 cells. Finally, in order to know the underlying mechanism for the observed species-specific response of SR-BI to probucol, we tested the following host-swap experiment. Probucol did not up-regulate human SR-BI protein in the liver of BAC transgenic mice lines carrying the entire human SR-BI genome. These results indicated that probucol stabilizes hepatic SR-BI protein possibly through species-specific mechanism.

PAT PROTEINS PERVADE LIPID BODY CORES AND ARE TRANSFERRED TO LIPID BODIES AT THE CELL MEMBRANE

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Lipid body-associated proteins of the PAT family – named after perilipin, adipophilin and TIP47 – are implicated in lipid body formation and lipid transport in fat related cells. How PAT proteins interact with lipids, lipid bodies and cell membranes is presently poorly understood. These proteins are generally considered to be restricted to the surface of lipid bodies, but recently we detected caveolin-1 inside lipid droplets in smooth muscle cells (Robenek et al. FASEB J 2004;18:866-8). Now, using freeze-fracture immunocytochemistry we found that the PAT proteins likewise completely pervade the cores of lipid bodies of cultivated macrophages and adipocytes. At the cell surface adipophilin and perilipin localize to discrete patches in the cytoplasmic leaflets of the plasma membrane. Colabeling studies indicated that the patches also contain TIP47. After incubating macrophages with acLDL for 24 h, labeling of PAT proteins in the plasma membrane is concentrated in raised, smooth-edged bumps. Underneath the membrane bumps and apposed to the plasma membrane are lipid bodies. In contrast to caveolin-1, labeling of PAT proteins is absent on ER membranes. We conclude that PAT proteins may be transferred to lipid bodies from the plasma membrane. DFG SFB 492.

LOW-DENSITY LIPOPROTEIN POTENTIATES THE PROATHEROGENIC ACTIVITY OF C-REACTIVE PROTEIN IN HUMANS

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Background: Clustering of elevated CRP and high LDL cholesterol correlates with a profound rise in cardiovascular risk. Recent evidence suggests that CRP itself elicits direct pro-atherogenic changes within the vascular wall, comprising endothelial dysfunction and increased atherothrombosis. To elucidate whether and to what extent LDL potentiates the pro-atherogenic effects of CRP, we assessed vascular reactivity in response to CRP exposure under normo- and hypercholesterolemic conditions in humans.
Methods: Forearm blood flow (FBF) responses to the endothelium-dependent and
–independent vasodilators serotonin (5HT) and nitroprusside (SNP), respectively, were measured in six patients with familial hypercholesterolemia (FH) and six normolipidemic controls before and 6 hours after recombinant human CRP (rhCRP) infusion (1.25 mg/kg) using venous occlusion plethysmography.
Results: At baseline, 5HT-induced vasodilation was impaired significantly in FH-patients (max 89.2±30.0% versus 117.7±13.1% in controls; $p<0.05$). Plasma CRP-levels increased after rhCRP-infusion to 30.0±3.5 mg/L and 26.2±2.9 mg/L in FH-patients and controls, respectively. Upon rhCRP-infusion 5HT-induced vasodilation decreased dramatically (max 42.3±25.7 %; $p=0.029$) in FH-patients, whereas no vaso-active effects were seen in controls. Endothelium-independent vasodilation remained unaltered throughout the study protocol.
Conclusion: These data reveal a pro-atherogenic synergy between CRP and LDL, resulting in a potent attenuation of endothelium-dependent vasodilation. The latter is likely to contribute to the CRP-associated risk increase particularly in hypercholesterolemic subjects. The present findings underscore that CRP is not only a diagnostic tool, but also an attractive therapeutic target in cardiovascular prevention strategies.

A NOVEL INHIBITOR OF OXIDOSQUALENE CYCLASE DECREASES VLDL AND LDL APOB100 THOUGH DECREASED VLDL PRODUCTION AND ENHANCED LDL CATABOLISM.

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Inhibition of 2,3 oxidosqualene: lanosterol cyclase (OSC), an enzyme in the cholesterol synthesis pathway, has the unique ability to inhibit cholesterol synthesis while simultaneously enhancing oxysterol synthesis. Recently, we demonstrated that this dual mechanism of action resulted in a dramatic inhibition of macrophage foam cell formation *in vitro* (Rowe et al Circ Res 2003;93:717-25). The objectives of the present study were to determine if a novel inhibitor of OSC would decrease LDL cholesterol and to define the mechanism(s) involved by examining apoB kinetics. ApoB kinetic studies were carried out in miniature pigs after 21 days treatment with 3 mg/kg/d of RO 0717625 or placebo (n=6/group). Pigs were fed a diet containing fat (35% of energy) and cholesterol (C) (400 mg/d). RO 0717625-treatment decreased plasma concentrations of total-C (-20%, $p<0.007$) and LDL-C (-29%, $p<0.005$). Other lipids were not affected significantly. ApoB kinetic parameters were determined using SAAMII following a bolus injection of trideuterated-leucine. VLDL apoB pool size decreased by 22% ($p<0.02$), due entirely to a decrease in VLDL production rate (-43%, $p<0.02$). LDL apoB pool size was decreased by 19% ($p<0.016$) due to a 47% ($p<0.03$) increase in the fractional catabolic rate (FCR). Other kinetic parameters were not affected. The increase in LDL apoB FCR was associated with a 2-fold increase in hepatic LDL receptor mRNA ($p<0.04$). Treatment significantly decreased hepatic total cholesterol (-16%, $p<0.045$) and cholesteryl ester (-39%, $p<0.025$). We conclude that a novel OSC inhibitor, RO 0717625, decreased VLDL and LDL apoB100 through decreased production and upregulation of LDL clearance. Thus, OSC is a potential therapeutic target for dyslipidemia and atherosclerosis.

TRANSCRIPTIONAL CONTROL OF ABCA1 AND HDL METABOLISM

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The ATP-binding cassette transporter ABCA1 is well known to be critically involved in cellular cholesterol and phospholipid efflux and HDL pool size. Mutations of ABCA1 have been causatively linked to familial HDL deficiency syndromes including Tangier Disease. Transcriptional regulation of ABCA1 by cellular cholesterol and cAMP was shown to be mediated by a LXR/RXR, Sp1, Sp3 and E-box proteins. In addition, there is upcoming evidence that a novel repressor-dependent regulatory network exists, which plays a pivotal role in controlling the expression of many important genes involved in HDL metabolism. This complex regulatory structure includes ZNF202 as well as other SCAN domain containing proteins, such as SDP1.

The zinc finger protein ZNF202 is a transcriptional repressor that binds to promoter elements predominantly found in genes involved in HDL and triglyceride metabolism, but also found in key genes of glucose turnover and growth, differentiation and signaling. We have shown that ZNF202 suppresses ABCA1 and ABCG1 gene expression via binding the universal corepressor KAP1. Furthermore, we could demonstrate that ZNF202 expression is inversely correlated with ABCA1, ABCG1 and apoE during monocyte differentiation and foam cell formation, reflecting a direct regulatory interdependence.

In addition, nuclear transloaction of ZNF202 by interaction with SCAN domain proteins such as SDP1, provides a further level of control for ABCA1, ABCG1, and apoE gene expression. SDP1 which functions as a PPARγ2 coactivator blocks binding of the universal corepressor KAP1 to ZNF202 and thereby can force expression of ZNF202 target genes. Therefore, the balanced expression of ZNF202 and SDP1 is required to control transcriptional regulatory networks within the HDL pathway, as well as in triglyceride and glucose metabolism.

GENETIC FACTORS CONTRIBUTING TO CHOLESTEROL ACCUMULATION AND ATHEROSCLEROSIS

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Hypercholesterolemia is the primary risk factor for development of coronary atherosclerosis. Plasma cholesterol levels are determined by relative rates of dietary cholesterol absorption, of plasma cholesterol removal and of cholesterol excretion into bile. We have taken a variety of genetic approaches to identify both rare and common sequence variations contributing to differences in plasma cholesterol levels, including expression arrays, positional cloning, candidate gene sequencing, and genetic association studies in a large multiracial, well-characterized population. Our most recent discoveries of new genetic defects contributing to inter-individual differences in plasma levels of cholesterol will be discussed.

DRUG-INDUCED METABOLISM OF CHOLESTEROL AND BILE ACIDS

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We have recently shown that patients treated with certain antiepileptic drugs, such as carbamazepine, have highly elevated levels of 4β-hydroxy-cholesterol in the circulation. Treatment of patients with gallstone disease with ursodeoxycholic acids for three weeks resulted in a 45 % increase in plasma 4β-hydroxycholesterol. Both carbamazepine and ursodeoxycholic acid induce CYP3A4 and it was shown that CYP3A4 is able to catalyze the conversion of cholesterol into 4β-hydroxycholesterol. This cholesterol metabolite is interesting since it has been reported to activate the nuclear receptor LXRα, a major regulator of lipid metabolism. Lipid anomalies are frequently reported side effects following antiepileptic drug treatment. In search for other endogenous substrates for CYP3A4 we found that this enzyme metabolized bile acids to metabolites with previously unknown origins. Thus, many bile acids were converted into 1β- and 22-hydroxylated metabolites. Patients treated with carbamazepine had a significantly increased urinary excretion of 1β-hydroxy bile acids. The most surprising finding was that CYP3A4 catalyzed the formation of 3-oxo bile acids. We propose a mechanism for the formation of 3-oxo bile acids involving a geminal diol, which is subsequently dehydrated to a ketone. 3-Oxo bile acids have been shown to be ligands for the nuclear receptors vitamin D receptor and pregnane X receptor both of which control the activity of CYP3A4. Formation of 3-oxo bile acids may thus enhance bile acid metabolism and thereby contribute to a protective effect in connection with cholestasis.

CHOLESTEROL EFFLUX POTENTIAL OF SERA FROM SUBJECTS WITH LCAT DEFICIENCY

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We have identified 9 Italian families with LCAT deficiency. In homozygotes (n=9) plasma HDL-C concentration was markedly reduced (11.5±7.5mg/dl) as well as plasma apoA-I (45.3±15.2mg/dl). The analysis of HDL size showed a predominance of small HDL_3 particles, with a great proportion of pre-beta HDL. Heterozygotes (n=22) have slightly reduced HDL-C and apoA-I levels (39.8±11.4mg/dl and 108.4±23.3mg/dl), with a significant increase (50%) in pre-beta HDL. Two models of cholesterol efflux were used to evaluate the contribution of ABCA1-dependent and -independent pathways: 1) Fu5aH hepatoma cells, expressing high levels of SR-BI and low levels of ABCA1, and 2) J774 macrophages expressing high levels of ABCA1, upon treatment with cAMP, and low levels of SR-BI. In Fu5AH cells, cholesterol efflux to sera from the homozygotes was significantly reduced by 45% compared with heterozygotes and control sera, which were not different. This result is consistent with the correlation between SR-BI-mediated efflux and HDL-C content of sera (r^2=0.6610). ABCA1-mediated efflux of the different sera was increased by 56% in heterozygotes and 106% in homozygotes compared to control sera, and was related with the percentage of pre-beta HDL (r^2=0.354), suggesting that despite the dramatic hypoalphalipoproteinemia, LCAT deficient homozygotes have efficient HDL. Whether the high efficiency of these HDL for cell cholesterol uptake would result in an improved reverse cholesterol transport remains to be established.

DEFICIENT BILE ACID SYNTHESIS IN HYPERLIPIDEMIC MICE ENHANCES HDL- CHOLESTEROL VIA INCREASED HEPATIC ABCA1 ACTIVITY

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HDL promotes reverse cholesterol (Chol) transport and plays thereby a crucial role in protection against development of atherosclerosis. The aim of this study was to evaluate the effect of impaired bile acid synthesis on HDL metabolism in mice on a mild hyperlipidemic background. Hereto, *cyp7a1*-knockout mice, which lack cholesterol 7α-hydroxylase, were cross-bred with *APOE*3-Leiden (E3L)* mice, a well-established mouse model for hyperlipidemia and atherosclerosis. *Cyp7a1*-deficiency resulted in a 74% decreased fecal bile acid excretion (P<0.001), and a 2-fold increased fecal neutral sterol excretion (P<0.005). Concomitantly, the hepatic content of Chol esters was increased (2.0-fold, P<0.05). Interestingly, *cyp7a1*$^{-/-}$*.E3L* mice showed highly elevated plasma HDL levels as compared to *E3L* littermates, including both HDL-Chol (56%, P<0.05), apoAI (37%), and apoAII (64%, P<0.05). To elucidate the mechanism underlying these effects, the effect of *cyp7a1*-deficiency on HDL clearance and production were determined. The activities of plasma factors involved in HDL metabolism, *i.e.* PLTP and LCAT, were not altered. Kinetic studies with [^{3}H]Chol oleate (CO)-labeled human HDL showed that the hepatic HDL clearance was not affected by *cyp7a1*-deficiency. Since *cyp7a1*-deficiency did increase the hepatic Chol content, we reasoned that the hepatic HDL synthesis may be enhanced by increased ABCA1 expression. Hepatic gene expression profiling by real-time PCR indeed demonstrated a 2.0-fold increase in *abca1* mRNA levels (P<0.05). To evaluate the effect of increased ABCA1 expression on hepatic Chol flux to HDL, [^{3}H]CO-labeled chylomicron-like emulsion particles were injected. After rapid uptake by the liver (t½ ~5 min), [^{3}H]Chol was liberated and secreted back into plasma in a time-dependent manner. In line with an increased ABCA1 expression, *cyp7a1*-deficient mice showed an increased appearance of ^{3}H-activity in HDL (37% at 8 h, P<0.05).
We conclude that the reduced bile acid synthesis in *cyp7a1-/-.E3L* mice leads to increased plasma HDL levels as a result of increased ABCA1 activity. These data thus confirm the major impact of hepatic ABCA1 on determination of HDL-Chol levels and underscore the close relationship between bile acid biosynthesis and HDL levels.

REGULATION OF BILE ACID SYNTHESIS IN HUMANS: ROLE OF THE HEPATIC EXPRESSION OF NUCLEAR RECEPTORS

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Bile acid synthesis plays a key role in cholesterol homeostasis. Recent data have highlighted the role of nuclear receptors in the transcriptional regulation of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme, in cellular and animal models. AIM of the present study was to analyze the expression of CYP7A1 and the possible correlations with related nuclear receptors in human livers. METHODS. Surgical liver biopsies were obtained in 32 patients; 12 with untreated gallbladder gallstones; 10 with abdominal cancer; 10 treated with cholestyramine (2), CDCA (3), or UDCA (5). mRNA levels of CYP7A1 and related nuclear receptors and coactivators were assayed by real-time quantitative RT-PCR. Serum levels of 7α-hydroxycholest-3-one (7α-3-ONE), a marker of bile acid synthesis, were assayed by GC-MS. RESULTS. CYP7A1 mRNA varied over a wide range. In gallstone patients PGC-1 was significantly ($p < 0.05$ on a log scale) less expressed in gallstone subjects; other genes were unaffected. In untreated patients serum levels of 7α-3-ONE showed an inverse correlation trend with age ($r = -0.48$, $p = 0.05$); no such correlation was present with CYP7A1 or other gene expression. Stepwise regression analysis with CYP7A1 mRNA levels as the dependent variable showed the strongest correlation with HNF-4 as the independent one ($r = 0.471$ on a log scale, $p < 0.05$), all other genes (including SHP) bringing non-significant further contribution. CONCLUSIONS. HNF-4 might play a relevant role in the regulation of CYP7A1 transcription in humans; no evidence for a relevant regulatory role of SHP, which was well documented in cellular models, is present. For the comprehensive understanding of CYP7A1 regulation in humans, post-transcriptional and post-translational control should also be considered. GRANT SUPPORT. Supported by 5th Framework Program grant QLGI-CT-2001-01513 and by COFIN-PRIN grant 2002062991.

DUAL REGULATION OF STEROL 27-HYDROXYLASE IN RESPONSE TO INFLAMMATORY STIMULI

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There is a link between inflammation and diseases characterized by abnormal lipid storage. Sterol 27-hydroxylase (*CYP27*) plays a dual role in cholesterol metabolism. In the liver it participates in the late steps of bile acid synthesis, in macrophages it initiates the conversion of cholesterol to more polar metabolites that are less prone to be stored within the cell. Therefore the level of *CYP27* expression may have important effects on cholesterol loading and, in turn, on the inflammatory potential of macrophages. The aim of our study was to explore the role of TNF-α on *CYP27* transcription in different cell lines.
We found that tumor necrosis factor α (TNF-α) stimulates the expression of NF-κB responsive genes as well as that of *CYP27* in PMA-differentiated THP-1 cells. However the promoter activity is not affected by TNF-α in undifferentiated THP-1 that are characterized by low level of *CYP27* transcription. These results suggest that TNF-α may potentiate *CYP27* transcription only in differentiated macrophages. In contrast we found that *CYP27* promoter is downregulated by TNF-α in liver-derived cells. We tested the hypothesis that the effect of TNF-α on *CYP27* promoter be mediated by NF-κB and we found that although overexpression of p65 represses *CYP27* promoter activity, the effect does not map to the same sequence involved in the response to TNF-α. These findings suggest that in liver cells TNF-α may affect *CYP27* transcription through a NF-κB-independent pathway.
Collectively our results indicate that *CYP27* gene is responsive to inflammatory stimuli but that the effect may be dependent on the cellular context. A further comprehension of the regulation of this gene may help understand its function in liver and in extrahepatic tissues.
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INCREASED FECAL NEUTRAL STEROL LOSS UPON PHARMACOLOGICAL ACTIVATION OF THE LIVER X RECEPTOR IS INDEPENDENT OF BILIARY CHOLESTEROL SECRETION

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Background: Reverse cholesterol transport (RCT) is usually defined as HDL-mediated flux of excess cholesterol from peripheral cells to the liver, followed by secretion into bile and disposal *via* the feces. Pharmacological activation of the liver X receptor (LXR), which controls genes crucially involved in RCT, leads to elevated HDL levels, enhanced biliary cholesterol secretion and increased fecal cholesterol loss in mice. In this study, we investigated the role of the intestine in enhanced fecal neutral sterol excretion upon activation of LXR. **Methods and results:** To segregate the role of biliary *vs.* directly intestine-derived cholesterol in reverse cholesterol transport, wild-type and Mdr2 P-glycoprotein deficient mice (*Mdr2*$^{-/-}$), which are unable to secrete cholesterol into bile, were treated with the synthetic LXR agonist GW3965. LXR activation increased HDL levels in both strains. Treatment with GW3965 increased biliary cholesterol secretion by 74 % in wild-type mice, but failed to induce this pathway in *Mdr2*$^{-/-}$ mice. As anticipated, LXR activation increased fecal neutral sterol excretion by 2.1-fold in wild-type mice. Surprisingly, an identical increase (2.1-fold) was observed in *Mdr2*$^{-/-}$ mice upon LXR activation. Intestinal gene expression of *Abca1*, *Abcg1*, *Abcg5* and *Abcg8* was strongly induced upon LXR activation in both strains, while expression of the gene encoding HMGCoA reductase, controlling cholesterol synthesis, remained unaffected. To examine whether the excreted cholesterol originate from the plasma department, [^{3}H]cholesterol was injected intravenously and secretion into the feces was followed for 4 days. Treatment with GW3965 increased the recovery of plasma-derived [^{3}H]cholesterol in the neutral sterol fraction of feces by 66% in *Mdr2*$^{-/-}$ mice. **Conclusions:** These data indicate that increased fecal cholesterol loss upon LXR activation is independent of biliary cholesterol secretion in mice. We propose that an important part of excess cholesterol is excreted directly via the intestine upon LXR activation, supporting the existence of an alternative, quantitatively important route for cholesterol disposal.

CLINICAL TRIALS WITH STATINS: WHAT HAVE WE LEARNT?

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Lowering LDL-cholesterol levels with a statin produces substantial reductions in the risks of heart attacks, ischaemic strokes and revascularisations (i.e. "major vascular events") among a wide range of high-risk individuals. Typically, lowering LDL-cholesterol by about 1 mmol/l reduces the rates of such major vascular events by about one-quarter irrespective of the blood lipid concentrations when treatment is initiated, and larger absolute reductions in LDL-cholesterol produce larger proportional reductions in risk. These benefits are additional to those of other treatments (such as aspirin, beta-blockers, angiotensin converting-enzyme inhibitors, and other antihypertensive therapy) shown to be beneficial for such high-risk people. Statin treatment has also been shown to be safe and well-tolerated. The absolute benefits of statin therapy depend chiefly on an individual's overall risk of major vascular events, rather than on their blood lipid concentrations alone (and more prolonged therapy should produce bigger absolute benefits). Consequently, statin therapy should now be considered routinely for all patients at sufficiently high risk of major vascular events, irrespective of their initial cholesterol levels, age or sex.

PLEIOTROPIC EFFECTS OF LIPID LOWERING AGENTS

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It is an expected property of a biologically active molecule to have multiple effects. These pleiotropic effects may account for desirable as well as undesirable properties. Typically, drug discovery focuses on one target aiming at a limited therapeutic outcome. Even though, pre-clinical studies may help predict some of them, many pleiotropic effects are discovered belatedly or unexpectedly when a drug reaches the clinic. In recent years, better awareness of these effects and of their potential has resulted in a large body of research, aimed at teasing them out for safety purposes, new applications or marketing advantages. This may be exemplified by looking at fibrates and statins. Most of the pleiotropic effects of fibrates are mediated through an interaction as ligands for the PPARα heterodimeric nuclear receptor and transcription factor, leading to enhancement of reverse cholesterol transport and triglyceride lowering. These pleiotropic effects extend now well beyond the lipid realm to molecules involved in inflammation and fibrinolysis. Similarly, most of the pleiotropic effects of statins are related to the prenylation of Rho and other small G-proteins. A wealth of information has accrued leading to the discovery and understanding of beneficial cardiovascular (anti-atherogenic and cardio-protective) and non-cardiovascular effects (arthritis, multiple sclerosis, Alzheimer's disease). They include improvement of endothelial dysfunction, anti-oxidant, anti-inflammatory, anti-coagulant, profibrinolytic, and anti-proliferative properties resulting in plaque stabilization and reduction of ischemic burden. Endothelial progenitor cell recruitment, immuno-modulation, inhibition of myocardial damage and antiarrhythmic properties contribute to the benefit. These properties may have far reaching clinical implications and help our understanding of mechanisms of drug actions and interactions. They may lead to new clinical indications or new drug development.

THE CURRENT AND FUTURE MODULATION OF HDL-C LEVELS

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In several epidemiological studies, decreased HDL cholesterol levels (hypoalphalipoproteinemia) have consistently been shown to be strongly associated with an increased risk of developing CAD. Particularly emphazised in prospective studies such as the Framingham Heart Study, and the Framingham Offspring study, individuals with low HDL cholesterol have been shown to have an elevated risk of developing CHD.
In some populations, a decreased level of HDL cholesterol has been the best discriminator between subjects with CAD and controls. Therefore, it is crucial that we obtain a better understanding of factors which contribute to this phenotype. Also in view of the fact that pharmacological intervention of low HDL cholesterol levels has, up till now, proven unsatisfactory and are essentially non-existent, it is important to understand the factors that regulate HDL-C levels in the circulation as this understanding will undoubtedly open up new avenues for the development of novel therapeutics.
In fact the elucidation of the role of cholesterylester; transferprotein (CETP) in reverse cholesterol transport, has led to the assessment of CETP inhibitors in clinical trials.
Both genetic and environmental factors may contribute to hypoalphalipoproteinemia. Several genetic causes for reduced HDL cholesterol have recently been identified.
In addition, in humans a large percentage of the variation in HDL cholesterol levels, remains unexplained. Thus, many genes involved in the metabolism of HDL cholesterol are as yet unknown.
In our attempt to identify novel genes controlling HDL-C levels, we have taken a family-based whole-genome linkage approach. We analyzed subjects of families, in which an autosomal dominant segregation of the familial hypoalphalipoproteinemia (FHA) or hyperalphalipoproteinemia (HALP) phenotype (defined by HDL-C levels below 5th or above the 95th percentile, respectively) was noticed.
In total, 2300 individuals from 135 FHA, and 1700 subjects from 170 HALP families were enrolled in this study. A dominant inheritance was noticed in 89 (66%) FHA and 110 (64%) HALP families. By means of linkage analysis in the families of two Tangier patients, we provided direct evidence that defects in the ATP binding Cassette A1 (ABCA1) were causal for FHA. ABCA1 mutations were found in two additional families. ABCA1 does play a pivotal role in cellular cholesterol efflux and atherosclerosis; comparison of mean carotid arterial wall thickness in carriers and non-affected family revealed that carriers of defects in ABCA1 are at increased risk for CAD.
In 6 FHA families we identified a novel apoA-I (L178P) mutation, which was found to cause severely disturbed HDL metabolism, endothelial dysfunction, increased arterial wall thickness and premature coronary artery disease.
Lastly, novel mutations were identified in the genes encoding for LCAT (T123I and V309M in 2 of the FHA families) and in CETP (IVS7+1; one of the HALP families). The biochemical and cardiovascular consequences of these defects are currently under investigation.
These findings show that increasing levels or activity of apoAI, ABCAI and LCAT might have beneficial consequences whereas the opposite is true for CETP. In this case inhibition might confer CAD protection.

APO A-I MILANO AND THE NEW ERA OF HDL THERAPY

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The apolipoprotein AI_{Milano} (AIM) was a chance discovery in an individual with extremely reduced HDL cholesterolemia (7-10 mg/dl). This observation, dating back 30 years, was accompanied by similar findings in two of the proband's children as well in his father. The family appeared to be remarkably free of vascular disease. Extensive studies led to the identification of a Cys for Arg replacement at position 173 of Apo A-I, thus identifying the first mutant of human apolipoproteins. A population study in Limone sul Garda eventually confirmed the suggestion that this might be a protective mutation. The 25 following years provided some important further insight, ie that the dimeric form has a prolonged permanence in blood and a high capacity to remove tissue cholesterol, in addition to attractive ancillary properties (antiinflammatory, profibrinolytic, etc)

HDL therapy is a novel area of therapeutic development. It attempts to improve the vascular benefit exerted by other agents, eg hypolipidemic drugs. Further, it takes advantage on novel techniques of coronary evaluation, potentially leading to non invasive diagnostic procedures.

HDL-liposomes containing AIM have induced rapid regression of atheromas in animals (Chiesa et al, Circ Res 2002; 90: 974) and in patients with established coronary disease (Nissen et al, JAMA 2003; 290: 2292). In this latter study, 5 weekly infusions of AIM (total dose as low as 5 g) have led to a better atheroma reduction than in the REVERSAL Study after 18 months of statin administration (Nissen et al, JAMA, 2004; 291: 1071).

The mechanism of this effect is likely to be associated to the powerful cholesterol removing capacity of the mutant. These observations have led to a number of attempts to develop alternatives that may mimic HDL, eg large unilamellar vesicles (LUV), phospholipid liposomes whose antiatheromatous activity has been preliminarily reported, as well as analogues of the apo AI amphipathic helices; d-isomers of these helices have produced inconstant findings in animals.

A major area of future research will be the development of non invasive methodologies for evaluating in particular coronary atheromas, in order to provide earlier HDL therapy. In case these new diagnostic modalities become available, then HDL therapy will move from the treatment of severe disease to the early management of initial lesions.

THE ROLE OF NON-HDL-C, APO B AND OTHER BIOMARKERS IN RISK ASSESSMENT

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There is increasing interest in the use of non-HDL-density lipoprotein (non HDL-C) cholesterol as a marker of coronary heart disease risk. Non-HDL-C provides a measure of all apolipoprotein B-containing lipoproteins, including very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL (including small, dense LDL), and lipoprotein(a), all of which have the potential to deliver cholesterol into the arterial wall. Non-HDL-C thus reflects atherogenic risk not captured by LDL cholesterol (LDL-C) measurement alone, particularly in the context of elevated triglycerides (TG) - a setting in which there are increased concentrations of VLDL and atherogenic VLDL remnants. In patients requiring lipid-lowering therapy, non-HDL-C is currently recommended for use as a secondary outcome measure, after LDL-C, in patients with elevated TG. Statins are the most effective agents for reducing levels of atherogenic lipoproteins. Consistent with its distinct pharmacologic properties compared with earlier members of the statin class, rosuvastatin potently reduces LDL-C levels and produces marked improvements in atherogenic lipid profiles at a low dose across a wide range of dyslipidemic phenotypes and patient subpopulations. A recent randomized, double-blind, crossover trial assessed the effects of rosuvastatin 40 mg in hypercholesterolemic patients with normal triglyceride levels (type IIa dyslipidemia) or elevated triglyceride levels (type IIb dyslipidemia). In hypertriglyceridemic patients, rosuvastatin reduced $VLDL_1$ by 46%, $VLDL_2$ by 42%, IDL by 54%, LDL by 58%, and small, dense LDL by 69%. In normotriglyceridemic patients, who have lower VLDL and remnant concentrations, rosuvastatin reduced $VLDL_1$ by 18%, $VLDL_2$ by 26%, IDL by 57%, LDL by 52%, and small, dense LDL by 44%. These studies reveal that rosuvastatin substantially corrects the atherogenic lipoprotein profile across a wide range of dyslipidemic phenotypes even when elevated concentrations of atherogenic lipoproteins are present as VLDL.

PLAQUES AND LIPIDS – MECHANISMS FOR CARDIOVASCULAR EVENT REDUCTION

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The acute coronary syndromes (ACS) – unstable angina and myocardial infarction (MI) – result from superficial erosion or rupture of the fibrous cap overlying an atherosclerotic plaque and resultant thrombus formation. Unstable lesions consist of a large lipid core, abundant inflammatory cells and a thin fibrous cap. Dyslipidaemia is a major factor in promoting the development of unstable plaques. In particular, elevated levels of small, dense LDL cholesterol (LDL-C) may promote atherogenesis. The small size of the particles enhances their transport across the endothelium, while their susceptibility to oxidation (oxLDL) facilitates their uptake by macrophages where they contribute to the lipid core of the plaque. The presence of small, dense LDL is directly associated with abundance of macrophages in the plaque. Moreover, the presence of oxLDL also doubles the expression of the metalloproteinases that break down the fibrous cap of the plaque. In contrast to the deleterious actions of oxLDL on atherosclerotic lesions, HDL cholesterol (HDL-C) helps to promote plaque stability. In addition to its role in reverse cholesterol transport, HDL-C can inhibit oxidation of LDL-C and block metalloproteinase expression. Studies of atherosclerotic plaques in patients undergoing revascularisation procedures have found that statin therapy is associated with a decrease in plaque volume and an increase in the amount of dense, fibrous tissue, as well as reductions in lipid content, in macrophage numbers and in levels of oxLDL and metalloproteinases. In order to maximize the benefits of statin therapy for plaque stabilization, a drug that both effectively lowers LDL-C (small, dense LDL specifically), and also raises HDL-C would be the ideal choice. Rosuvastatin is highly effective in reducing LDL-C, and specifically the cholesterol in the small, dense, LDL particles (highly susceptible to oxidation), particularly in patients with the common atherogenic lipid phenotype characterized by low HDL-C, mild hypertriglyceridaemia and prevalence of small, dense LDL (i.e. Metabolic Syndrome). Furthermore, rosuvastatin produces significant increases in HDL-C levels, which are maintained across the dose range. These data suggest that treatment with rosuvastatin, highly effective on lipid abnormalities associated the unstable atherosclerotic plaque, may prove to be very effective in enhancing plaque stability and so reducing the risk of ACS. Two ongoing clinical trials, ASTEROID, using intravascular ultrasound (IVUS) and quantitative coronary angiography (QCA), and ORION, using MRI and ultrasound, will provide evidence of the possible beneficial effects of rosuvastatin on plaque formation and stability.

OPTIMAL CARDIOVASCULAR RISK REDUCTION: LDL-C VERSUS APOLIPOPROTEIN TARGETS

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The recently released 'whitepaper' addition to the NCEP-ATPIII guidelines has further lowered the LDLC goals for treatment in specific higher risk CAD risk groups. The goals for LDLC depending on risk are now 70, 100, 130 and 160 mg/dL. NCEP ATP III also introduced for the first time the concept of nonHDLC, suggested using this parameter when triglycerides were between 200 – 500 mg/dL and set treatment goals for nonHDLC. The selection of nonHDLC 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. Minimal data currently exists comparing the efficacy of lipid-lowering therapies on achieving ApoB cut points of 90 and 105 mg/dL to these new LDLC and nonHDLC targets. Apo B could, with its more solid scientific and biological foundation, provide a far simpler, and potentially more sensitive and specific, measurement for clinicians to measure in all circumstances, and base therapeutic decisions on, than what has become a confusing array of lipid subfractions.

NCEP-ATP III also increased the emphasis on HDLC and raised to 40 mg/dL the cutpoint for determining additional CAD risk and using HDLC as a recommendation of treatment. However HDLC still remains one of the most methodologically challenged assays in the clinical laboratory with significant biases from laboratory to laboratory. Apolipoprotein A1 (Apo A1) provides a more biologically robust and analytically less demanding procedure. Again minimal if any data on Apo A1 compared to HDLC cutpoints and changes during on therapy exists.

Finally from both epidemiological and clinical trial data the best predictor of future risk for CAD appears to be the Apo B:Apo A1 ratio which could also provide a basis for future treatment goals.

GUIDELINES VERSUS CLINICAL PRACTICE: WHAT REALLY WORKS ?

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Current lipid guidelines in Europe and the US endorse the use of cholesterol targets. Statins, because of their excellent tolerability and their beneficial impact on the lipid profile, leading to significant reductions in the risk of cardiovascular events, have become the drugs of first choice to help achieve these targets. However, the scale of this problem across Europe means that for any management strategy to be effective, it must also be robust and simple. Rosuvastatin is a new statin with a range of highly favorable characteristics. These include powerful lipid lowering, low lipophilicity, high hepatocyte selectivity, and a low propensity for cytochrome P450 drug interactions. In comparative clinical trials, rosuvastatin given at 10 to 40 mg/day reduced low-density lipoprotein (LDL) cholesterol to a significantly greater extent than the same and some higher doses of pravastatin, simvastatin, or atorvastatin. In addition, rosuvastatin exhibits beneficial effects on other lipid parameters such as high-density lipoprotein (HDL) cholesterol and triglycerides. In particular, the HDL-cholesterol raising effects of rosuvastatin are maintained at higher doses, unlike those seen with some other statins. Rosuvastatin's safety profile has been demonstrated to be similar to those of other statins, and the drug is very well tolerated across the 10 to 40 mg dose range. With its greater LDL-cholesterol lowering, rosuvastatin will allow more patients to reach cholesterol goals including the new European Guidelines and NCEP ATP III –even at the starting dose. This, taken together with the good patient tolerability of rosuvastatin and its once daily dosing regimen at any time, with or without food, offers the clinician a very simple, but highly effective strategy for patient management.

MECHANISMS OF PLAQUE INSTABILITY

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It is well recognised that atherogenic stimuli like hypertension, hypercholesterolemia, smoking and diabetes cause endothelial dysfunction followed by chemo-attraction of inflammatory cells which then migrate in the subendothelium and originate the atherosclerotic plaque. It is likely that both the intensity of atherogenic stimuli and the reactivity of inflammatory cells play a key role in the transition from endothelial dysfunction to plaque formation. This contention is supported by the observation that in asymptomatic subjects long-term risk of major cardiovascular events conferred by raised serum levels of C-reactive protein (CRP), a prototypic marker of inflammation, and by the presence of traditional risk factors is additive. In a sizeable proportion of patients the sudden transition from the asymptomatic or stable phase of coronary atheroscleorsis to acute coronary syndromes is associated to an inflammatory outburst. The sudden activation of inflammatory cells in the unstable plaque results in the release of cytokines which have the potential to cause endothelial activation, plaque fissuring and vasoconstriction followed by thrombus formation. Recent observations suggest the intriguing possibility that inflammation is not limited to the culprit stenosis, but it is widespread in the whole coronary circulation. The triggers of inflammation are still unknown, but recent evidence suggests that Chlamydia pmeumoniae-related immune mechanisms can play an important role. Regardless of triggers, the intensity of the inflammatory outburst associated to coronary instability, as assessed by measuring serum levels of CRP, is a powerful independent predictor of short-medium term outcome, even in patients submitted to an early invasive strategy. Finally, CRP is a marker of the "iatrogenic" inflammation caused by stent implantation. In this setting also, CRP predicts restenosis both in stable and unstable patients. Prospective studies are warranted in order to establish whether the prognostic information conveyed by CRP can improve treatment targeting both in primary and secondary prevention of ischemic heart disease.

IMMUNITY AND ATHEROSCLEROSIS

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Atherosclerosis is currently described as an inflammatory vascular disease, characterized by accumulation of lipids, fibrous elements and abundant immune cells, particularly macrophages and T cells. Identification of this immune/inflammatory infiltrate suggest the involvement of immune mechanisms in atherogenesis. During recent years, experiments in genetically altered mice have lent further support to the hypothesis that immune mechanisms contribute to atheroma formation. Cells and molecules that mediate both adaptive immunity, which depends on antigen-specific immunologic memory, and innate immunity, characteristically antigen- and memory-independent, localize in atherosclerotic lesions.

The innate immunity involves several different cell types, most importantly those of the mononuclear phagocyte lineage. Macrophages express receptors that recognize a broad range of molecular patterns foreign to the mammalian organism, also commonly found on pathogens, such as various scavenger receptors or Toll-like receptors. Ligation of these receptors (ie by oxidized LDL, LPS, HSP) activates NF-kB and MAPK pathways, leading to phagocytosis, apoptosis, production of reactive oxygen species, and leukocyte recruitment.

The principal molecular mediators of innate immunity include proteins commonly designated as cytokines, which have been associated with atherosclerosis.

The adaptive immunity involves T and B lymphocytes, as well as dendritic cells, and depends on the generation of large numbers of antigen receptors (ie, TCRs) and immunoglobulins. Initial activation of "naïve" T cells requires strong activating stimuli best provided by the dendritic cell. Once T cells recognize foreign antigens presented to them, they initiate adaptive immune responses. Cells bearing these antigens are attack by cytotoxic T lymphocytes, with stimulation of B cells to produce antibodies against these specific antigens, and induction of inflammation, with enhanced innate responses, in the area where the antigen is present. Although cells implicated in adaptive immune responses differ entirely from those of innate immunity, many pathways overlap to a great extent. For instance, activation of T cells can leads to secretion of interferon-g, which primes macrophages, or the production TNF-a, which can activate NF-kB. Activated T cells can also express CD40 ligand, which activates its receptor CD40 largely expressed on macrophages.

Number of evidence implicate the involvement of mediators of immunity in all stages of atherosclerosis, from the earliest stage of lesion development to the ultimate clinical

NEW BIOMARKERS FOR CARDIOVASCULAR DISEASE

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Identification of biomarkers associated with atherosclerotic cardiovascular disease can potentially be used clinically in at least three ways: risk stratification of patients to determine which therapy to initiate, surrogate markers of efficacy to assess response to therapy, and identification of targets of therapy causally related to atherothrombotic events. Biomarkers related to inflammation such as C-reactive protein (CRP) have been associated with increased risk for coronary heart disease (CHD) events, reflecting the role of inflammation in atherogenesis and atherothrombotic events. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a proinflammatory enzyme primarily associated with low-density lipoprotein (LDL). In the Atherosclerosis Risk in Communities study, in 12,819 apparently healthy middle-aged men and women, Lp-PLA_2 and CRP levels were higher in individuals who developed incident CHD or stroke than in noncases, and both Lp-PLA_2 and CRP were associated with incident CHD and stroke after adjustment for age, sex, and race. In a model adjusted for traditional risk factors including LDL-C, Lp-PLA_2 was associated with CHD only in the subgroup of individuals with LDL-C <130 mg/dL. For stroke, Lp-PLA_2 tertile remained predictive in a model including CRP, traditional risk factors, body mass index, triglycerides, and antihypertensive medication. Another inflammatory marker—monocyte chemoattractant protein–1 (MCP-1), which mediates the recruitment of monocytes to the vessel wall at sites of atherosclerosis—was significantly higher in ARIC participants with peripheral artery disease (PAD) than in controls, and was significantly associated with PAD independent of traditional CHD risk factors. Additional research is needed to determine the role of biomarkers related to inflammation for risk stratification, surrogate markers guiding therapy, and targets of therapy.

ANTI-INFLAMMATORY EFFECT OF STATINS ON THE INTIMAL HYPERPLASIA AFTER STENT IMPLANTATION: IMPLICATION FOR AGGRESSIVE INFLAMMATION MANAGEMENT IN CORONARY INJURY MODELS

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Recent evidence indicates that the pleiotropic effect of statins involve modulation of smooth muscle cell (SMC) proliferation, anti-inflammation, and anti-thrombotic action, which contribute to the prevention of restenosis following coronary intervention. We tested the hypothesis that cerivastatin-eluting stent or systemic administration of pitavastatin might inhibit the early inflammatory response, platelet deposition, and SMC proliferation, resulting in prevention of neointimal hyperplasia in a porcine coronary model.

Methods and Results. The pigs were divided into three groups, cerivastatin-loaded stents group, pitavastatin group, and control (bare-metal stent) group. Stents were deployed in the porcine coronary arteries. The level of total cholesterol did not differ between groups. Inflammatory cell infiltration and platelet deposition surrounding the stent struts was evaluated with scanning electron microscopy at day 3 and was significantly reduced in the treated vessels compared to control. Histomorphometry showed reduced neointimal area (1.74 ± 0.65 vs. 2.78 ± 0.28 vs. $3.77\pm0.33 mm^2$, $p<0.01$, cerivastatin, pitavastatin, and control, respectively) and increased lumen in treated arteries versus non-treated vessel despite similar injury scores. Furthermore, proliferaive intimal composition was assessed with special stains with quantitative image analysis. Intimal area from statin group had fewer SMC and inflammatory cells and higher collagen content.

In conclusion, cerivastatin coated stents and pitavastatin results in a significantly decreased neointimal hyperplasia with decreased inflammatory response and platelet deposition and modulation of the cell component in porcine coronary arteries. The pleiotropic effect of statin plays a crucial role on the process of restenosis after vascular injury.

DYSLIPIDEMIA-INDUCED INFLAMMATION AND ITS MODULATION BY STATIN

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Vasculitis and Atherosclerosis involve a complex process that is associated with vascular wall dysfunction. An increasingly large body of evidence points to a crucial role of leukocyte-endothelial interactions in atherosclerotic plaque formation. The adhesion of circulating monocytes to the intimal endothelial cell monolayer is thought to be one of the earliest events in naturally ocurring human and experimental animal models of atherosclerosis. Upregulation of the number and/or affinity of numerous adhesion receptors and counter-receptors expressed on both endothelial cells and leukocytes appears to be involved, including selectins, ICAM-1, VCAM-1, and β 1-and β 2-integrins. Our group has been focused on the elucidation and modulation of this process utilizing a simulated flow chamber system. Recently, we were able to show that triglyceride-rich remnant lipoprotein particle (RLP) significantly enhances monocytes adhesion to vascular endothelium. Moreover, HMG CoA reductase inhibitor (statin) modulates observed adhesion of RLP-induced monocytes. Underlined mechanisms of above phenomenon seemed to involve combined effects of RhoA inactivation, followed by reduced phosphorylation of focal adhesion kinase. Further, RLP treatment significantly induces proliferation of vascular smooth muscle cells via transactivation of EGF Receptor. These findings may point a potential inflammatory property of RLP in atherosclerosis.

MANAGEMENT OF CARDIOVASCULAR RISKS IN DIABETICS AND OTHER PATIENT POPULATIONS: WHAT'S THE VALUE OF NEW DUAL PPAR THERAPY FOR DIABETIC PATIENTS?

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Prevention and treatment of type 2 diabetes mellitus and the metabolic syndrome represent a major clinical challenge, because effective strategies such as fat restriction and exercise are difficult to implement into diabetes treatment. Over the past decade, the elucidation of key regulators of energy balance and insulin signaling have revolutionized our understanding of fat and sugar metabolism and their intimate link. The three 'lipid-sensing' peroxisome proliferator-activated receptors (PPAR-alpha, PPAR-gamma and PPAR-delta) exemplify this connection, regulating diverse aspects of lipid and glucose homeostasis, and serving as bona fide therapeutic targets. Dual-acting PPAR agonists are a novel group of compounds that activate nuclear transcription factors. By activating both PPAR-alpha and PPAR-gamma receptors, they simultaneously reduce atherogenic triglycerides, raise cardioprotective HDL levels (PPAR-alpha activity), improve insulin resistance (PPAR-gamma activity) and decrease vascular inflammation. Thus, they address many of the core features seen in people with metabolic syndrome and may help to reverse the underlying disease process and its adverse clinical sequels, which includes CVD and diabetes. Safe dual-acting PPAR agonists will be very useful to treat patients with type 2 diabetes and/or with the metabolic syndrome.

MANAGEMENT OF CARDIOVASCULAR RISK: THE VALUE OF STATIN THERAPY

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Cardiovascular risk factors include those which are intrinsic and fixed such as gender, family history and age, as well as modifiable factors such as smoking, unhealthy diet, lack of exercise and obesity. While lifestyle change is central to dealing with these, active pharmacological therapy is essential for patients at high risk of coronary heart disease (CHD). For high-risk patients the European Guidelines recommend that total plasma cholesterol level be 4.5 mmol/L or lower, with LDL cholesterol below 2.5 mmol/L. This is based on a massive body of evidence from large placebo-controlled clinical trials, all of which have shown the efficacy of statin therapy in reducing cardiovascular morbidity and mortality without an increase in the overall incidence of adverse effects.

It is sometimes overlooked that significant iatrogenic dyslipidaemia may result from the treatment of non-cardiovascular diseases. For example, second generation antipsychotic therapies can lead to metabolic syndrome and some protease inhibitors cause severe hypercholesterolaemia and hypertriglyceridaemia. Statin treatment should also be considered in such patients, with careful consideration of the drug interaction potential of the statin prescribed.

The long-term safety of statin therapy has been demonstrated in numerous large-scale trials including the Pravastatin Pooling Project (PPP). The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) showed that the benefits of pravastatin treatment extend beyond middle aged persons to elderly men and women with, or at high risk of developing, cardiovascular disease.

METABOLIC ISSUES IN PSYCHIATRIC DISORDERS

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Individuals with mental disorders suffer increased prevalence of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) compared to the general population. Several factors may account for this excess morbidity and mortality, including increased prevalence of overweight and obesity, high levels of smoking, and reported increases in the prevalence of hyperglycemia and dyslipidemia. Overweight and obesity, particularly increased adiposity, is associated with insulin resistance, dyslipidemia, hypertension, type 2 diabetes mellitus, and cardiovascular disease (e.g., risk for myocardial infarction and stroke). Treatment with medications that include certain antipsychotic drugs is associated with adverse metabolic effects that include substantial increases in adiposity. Weight gain is reported in up to 50% of patients receiving certain chronic treatments for schizophrenia. A range of evidence, including randomized clinical studies, suggests that these same antipsychotic drugs can increase risk of insulin resistance, hyperglycemia, dyslipidemia and T2DM. Primary and secondary prevention efforts aiming to reduce cardiovascular disease focus on well-known modifiable risk factors, specifically overweight and obesity, hypertension, hyperglycemia, dyslipidemia and smoking. Strategies to reduce risk and maximize benefits of treatment, including guidelines for monitoring of metabolic parameters, can be readily applied to the treatment of individuals with mental disorders to reduce the risk of T2DM and cardiovascular disease.

THE PHARMACOECONOMIC CONSEQUENCES OF ANTIPSYCHOTIC–RELATED METABOLIC EVENTS

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Extensive reports in the literature strongly suggest that metabolic sequelae such as obesity, dyslipidemia, glucose dysregulation, and metabolic syndrome derive from the use of certain atypical antipsychotic agents. This observation, coupled with the already elevated cardiovascular risk profile of schizophrenia patients[1], has serious prognostic and cost implications with regard to treatment-related diabetes and coronary disease incidence in this population. Using evidence derived from a number of epidemiologic studies, the recent ADA Consensus Panel has concluded that some agents are associated with greater diabetes risk than others.

This presentation will describe the prevalence of metabolic syndrome in both general and schizophrenic populations, its prognostic relevance and its exacerbation among schizophrenic patients treated with particular antipsychotic agents. Furthermore, the per patient costs associated with the treatment of metabolic syndrome, diabetes, and coronary heart disease in these populations is discussed.

Lastly, reference is made to the clinical and economic impact of metabolic adverse events and their consequences, particularly diabetes, which is considerable and warrants serious consideration by treating physicians.

HIV LIPODYSTROPHY AND DYSLIPIDEMIA: PATHOGENESIS

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Current treatment of HIV infection consists of highly active antiretroviral therapy (HAART), including nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI), and non-nucleoside reverse transcriptase inhibitors (NNRTI). HAART therapy has brought tremendous clinical benefits, but many patients develop body fat redistribution, dyslipidemia, and insulin resistance, a constellation termed HIV-associated lipodystrophy. There is accumulating data that lipodystrophy is associated with an increase in cardiovascular risk, as shown in the D:A:D and other studies.

Dyslipidemia had existed in the pre-HAART era and those abnormalities improved with NRTI therapy. In contrast, dyslipidemia is promoted by certain PIs in HIV-infected subjects and in normal volunteers. Some of the effect in HIV represents a return to normal, or to prior health status, rather than toxicity. The mechanism of action may be related to the inhibition of intracellular degradation of apoprotein B in the liver, with excess hepatic secretion of VLDL, as documented in *in vivo* studies.

However, an increase in VLDL secretion doesn't explain the whole picture. Some studies have documented a delay in lipid clearance from the circulation, as opposed to an increase in secretion. In addition, hypertriglyceridemia may develop in patients not receiving PI therapy, as shown in a study which compared the nucleotide, tenofovir, and the NRTI, d4T. The d4T treated group had a progressive rise in serum triglycerides over 3 years, a change that was seen in men but not in women. The d4T group also developed lipoatrophy, an alteration that has been linked to mitochondrial dysfunction in adipose tissue. It is possible that hypertriglyceridemia could occur as a result of mitochondrial dysfunction in liver, muscle, or adipose tissue.

Other antiretrovirals, specifically the NNRTI class, raise serum HDL-cholesterol concentrations. Other, concurrent therapies also may affect lipid metabolism, e.g., anabolic steroids, which decrease HDL-cholesterol and elevate in LDL-cholesterol. Finally, dyslipidemia may be related other, non-HIV related factors.

ACTIVATING THE PPAR RECEPTORS - BASIC SCIENCE AND CLINICAL RAMIFICATIONS.

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Cardiovascular diseases (CVD) remain the leading cause of mortality in developed countries. Several risk factors are associated with these pathologies including type 2 diabetes, obesity, insulin resistance, dyslipidemia and hypertension. Different pharmacological therapies have been developed to control the risk factors associated to CVD. PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily controlling lipid and glucose metabolism as well as inflammatory risk factors for CVD. PPARα agonists, such as the fibrates, correct dyslipidemia, thus decreasing CVD risk. PPARγ agonists, such as the glitazones, increase insulin sensitivity and decrease plasma glucose levels in diabetic patients. Moreover, both PPARα and PPARγ agonists exert anti-inflammatory activities in liver, adipose and vascular tissues. In this presentation, we will focus on the mode of action of PPARα and γ agonists, illustrating the potential interest of newly developed dual PPAR agonists in the treatment of the global risk profile of patients suffering from the metabolic syndrome or type 2 diabetes.

ADIPOCYTOKINES AND METABOLIC SYNDROME

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Clustering of multiple risk factors such as impaired glucose metabolism, lipid disorders and hypertension has been shown to be major background of atherosclerotic diseases and disease entities such as metabolic syndrome become popular as highly atherogenic state. Although each common risk may generally co-exist by accident in one individual, multiple risk factor clustering in metabolic syndrome does not occur by accident and there should be a key player for the syndrome. Visceral fat accumulation has been shown to cause impaired glucose metabolism, lipid disorders and hypertension, therefore visceral fat accumulation is considered to be a key player in metabolic syndrome. In order to clarify the mechanism of metabolic syndrome, we studied on the molecular characteristic of adipose tissue and adipocytes by investigating expressed genes in visceral and subcutaneous adipocytes and revealed that adipocytes, especially visceral adipocytes are secreting a variety of bioactive substances which we called adipocytokines. We demonstrated that visceral fat accumulation causes abnormalities of adipocytokine secretion such as hypersecretion of PAI-1 which is related to thrombogenic vascular diseases.

We discovered an important benign adipocytokine named adiponectin which protects against the development of diabetes mellitus, hypertension, inflammation and atherosclerotic vascular diseases. We also demonstrated that plasma levels of adiponectin decreased in the subjects with visceral fat accumulation, which results in the development of impaired glucose metabolism with insulin resistance, hyperlipidemia, hypertension in one individual.

In this lecture, I would like to show the molecular mechanism of metabolic syndrome with respect to visceral fat accumulation and the dyssecretion of adipocytokines especially focusing on adiponectin.

INSULIN RESISTANCE AND VASCULAR DISEASE: THE ROLE OF INSULIN BEYOND THE RISK FACTORS.

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It is known that hyperinsulinemia precedes type 2 diabetes and that it is associated with an adverse cardiovascular risk profile. Recent data suggest that hyperinsulinemia reflects a compensatory mechanism of decreased insulin sensitivity of the peripheral tissues to insulin. This "insulin resistance" might be essential in the pathogenesis of CVD. In vivo, in humans, locally elevated insulin levels significantly alter the endothelium-dependent vasodilation. Recent studies have reported that oxidative stress may play an important role in the pathogenesis of cardiovascular disease in patients with diabetes mellitus. There is evidence that hyperinsulinemia may be involved in the generation of oxidative stress. We recently assessed the capability of elevated insulin levels to generate free radicals in cultured fibroblasts. Insulin increased free radical production in sigmoidal fashion. To delineate the underlying mechanisms by which insulin generates free radicals, we assessed the translocation of p47phox, a subunit of NAD(P)H oxidase from cytosol to membrane. In the cells exposed to insulin NAD(P)H oxidase was significantly activated. We thus provide direct evidence that insulin generates free radicals in isolated human fibroblasts through the activation of NAD(P)H oxidase. These observations may offer an explanation for the atherogenic role of chronic hyperinsulinemia.

C-REACTIVE PROTEIN AND CARDIOVASCULAR RISK

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Cardiovascular disease (CVD) remains the leading cause of death in the United States and Europe despite advances in the diagnosis and management of the disease. Hyperlipidemia has proven to be one of the most important modifiable risk factors for CVD and reductions in cardiovascular morbidity and mortality can in part be attributed to cholesterol screening. However, screening studies indicate that a substantial proportion of cardiovascular events will occur in persons with normal cholesterol levels or one or less risk factors for CVD. Meanwhile, experimental evidence indicates that inflammation plays a critical role in the pathogenesis of atherosclerosis. To this end, multiple, large scale prospective studies have evaluated the role of several molecular/inflammatory markers in predicting the future risk of myocardial infarction and stroke including lipoprotein(a), D-dimer, oxidized LDL-C, homocysteine, fibrinogen, high-sensitivity C-reactive protein and tPA antigen. However, CRP has been the most well studied marker of risk and data demonstrate that CRP independently predicts future CVD. Furthermore, the combination of CRP and lipid screening appears to be a better predictor of CVD risk than lipid evaluation. Moreover, those individuals with high CRP levels but normal/low LDL cholesterol levels have a similar risk for CVD than those persons with hyperlipidemia. While, physical activity, diet, aspirin and statin drugs have been shown to decrease CRP levels, trials relating any reduction in CRP levels with attenuated CVD mortality are in progress.

11β-HSD1 INHIBITION AMELIORATES METABOLIC SYNDROME IN MICE.

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The key symptoms of the metabolic syndrome (MS) may be caused by high intracellular levels of glucocorticoid hormones. Chronic exposure to elevated circulating glucocorticoids can lead to metabolic changes which resemble those of MS, and features of the MS can be reversed by lowering systemic glucocorticoid levels or by treatment with a glucocorticoid receptor antagonist. 11β hydroxysteroid dehydrogenase-type 1 (11β-HSD1) is a widely expressed NADP(H)-dependent enzyme that acts primarily as a reductase, generating active glucocorticoids in intact cells from inactive precursors. Several lines of evidence suggest that 11βHSD1 acts physiologically to raise glucocorticoid tone and could thereby play an important role in MS. We report here that pharmacologic inhibition of HSD1 can also confer resistance to MS. High-throughput screening identified a novel potent and selective adamantyl triazole enzyme inhibitor of 11β-HSD1. The ability of the compound to inhibit enzyme activity in mice was confirmed by pharmacodynamic assessment after oral dosing. Administration of this compound caused a 7% body weight loss in diet induced obese mice after 10 days of dosing ($p<0.001$). Insulin levels were lowered 59% ($p<0.001$) in these animals while fasting glucose was lowered 15% ($p<0.001$). In mice fed a high fat diet and dosed with the pancreatic beta cell toxin streptozotocin to induce a type 2 diabetic phenotype, the HSD1 inhibitor lowered fasting glucose by 80% ($p<0.001$) and decreased serum glucose excursions in an IPGTT and OGTT after 9 days of treatment. Triglycerides were lowered by 58% and serum leptin and glucagon levels were lowered to control levels. We conclude that pharmacologic inhibition of 11βHSD1 can have a salutary effect on multiple aspects of MS.

ADIPOSE TISSUE FATTY ACID-HANDLING PROTEIN EXPRESSION, ADIPOSE TISSUE AND SERUM FATTY ACID COMPOSITION, AND MARKERS OF INSULIN RESISTANCE

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Insulin resistance is related to disturbed fatty acid (FA) metabolism in adipose tissue and other tissues, and characteristic serum lipid ester FA composition patterns are observed in insulin resistant states. Several proteins are involved in transmembrane and intracellular FA trafficking and activation of FAs via acyl-CoA formation prior to cellular metabolism.
Hypothesising that such FA-handling proteins play a physiological role with respect to FA metabolism and insulin resistance, the aim of this study was to investigate relationships between mRNA expression levels of FA-handling proteins (adipocyte lipid binding protein, keratinocyte lipid binding protein, fatty acid transport protein-1 and -4 (FATP1, FATP4), fatty acid translocase/CD36, plasma membrane fatty acid binding protein, and acyl-CoA synthase-1 (ACS1)) in subcutaneous adipose tissue, FA composition of the adipose tissue, serum non-esterified FA, and serum phospholipid compartments, and markers of insulin resistance, in a subset of 75 subjects recruited from a cohort of 294 Swedish 63-year-old men with a range of insulin sensitivities.
ACS1 expression levels were negatively correlated with measures of insulin resistance, and an opposite trend was observed for FATP4 expression levels. Furthermore, ACS1 and FATP4 expression levels correlated with FA composition variables of proposed relevance in relation to insulin resistance. In particular, ACS1 expression levels correlated negatively with the adipose tissue relative content of 16:0 (palmitic acid), and FATP4 expression levels correlated positively with the adipose tissue relative content of 20:4 n-6 (arachidonic acid), FA composition variables which were both positively correlated with homeostasis model assessment index in the current study (n=289), indicating that handling of 16:0 in adipose tissue by ACS1 and of 20:4 n-6 by FATP4 are quantitatively related to insulin sensitivity.
These findings suggest a physiological role of FA-handling proteins in relation to insulin sensitivity, via their involvement in FA trafficking and metabolism.

KINETIC CHARACTERIZATION AND SPECIFIC INHIBITION OF HORMONE-SENSITIVE LIPASE

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Hormone sensitive Lipase (HSL) is the rate limiting enzyme of intracellular triglyceride hydrolysis and mobilization in adipocytes. It is regulated by signaling pathways triggered by adrenalin, noradrenalin and glucagon and counter regulated by insulin. 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 and liver, where they are metabolized to acetyl-CoA to a certain degree. Thus, the inhibition of HSL might offer a pharmacological approach to reduce FFA levels and diminish peripheral insulin resistance. Pre-incubation of the recombinant human HSL with a serine esterase inhibitor such as diethyl *p*-nitrophenyl phosphate in 1 to 100 molar excess leads to complete HSL inhibition within 15 minutes. This result indicates that the catalytic serine of HSL is highly reactive and that it is be readily accessible. Similar behavior was also observed with lipases with no lid domain covering their active site. The 3-D structure of HSL, which still remains to be determined, may therefore lack the lid domain known to exist in various other lipases. We have developed selective nanomolar inhibitors of HSL. The inhibition of HSL is demonstrated under several assay conditions and a kinetic model is proposed to describe the inhibition of HSL by synthetic inhibitors in the aqueous phase as well as the reactivation process at the lipid-water interface.

THE METABOLIC SYNDROME IS GRADUALLY AND INDEPENDENTLY PREDICTIVE FOR VASCULAR EVENTS IN PATIENTS UNDERGOING CORONARY ANGIOGRAPHY

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Background: Prospective data on the metabolic syndrome (MS) in patients characterized by coronary angiography are scarce. We hypothesized that the MS is an independent predictor of vascular events in patients undergoing coronary angiography.
Methods: We enrolled 756 consecutive patients undergoing coronary angiography for the evaluation of suspected myocardial ischemia. According to ATP III criteria, we defined the MS as the presence of any three of: waist circumference >102 cm in men and >88 cm in women, triglycerides of 150 mg/dl or higher, HDL cholesterol <40 mg/dl in men and <50 mg/dl in women, blood pressure of 130/85 mmHg or higher, or fasting glucose of 110 mg/dl or higher.
Results: The HOMA index of insulin resistance increased significantly with an increasing number of MS risk factors (p for trend <0.001). Patients with MS (n = 280) were at an increased risk of significant coronary stenoses of 50% or more at baseline (adjusted OR = 1.81 [1.09-3.01]; p = 0.022). Prospectively, in Cox regression analyses adjusting for age, gender, significant coronary stenoses at baseline, smoking, body mass index, LDL cholesterol, and use of aspirin, of antihypertensive drugs and of lipid-lowering drugs, the MS significantly predicted vascular events (n = 95) during a follow-up period of 2.2 ± 0.5 years among men (OR = 2.12 [1.26-3.57]; p = 0.005) and women (OR = 5.52 [1.60-22.47]; p = 0.017), and among patients with diabetes mellitus type 2 (OR = 3.89 [1.12-3.52]; p = 0.033) as well as among non-diabetic patients (OR = 2.05 [1.13-3.71]; p = 0.018). Cardiovascular risk increased gradually with an increasing number of components of the MS (p for trend <0.001). After additional adjustment for HOMA insulin resistance the MS remained significantly predictive for the incidence of vascular events (OR = 2.26 [1.25-4.09]; p = 0.007).
Conclusions: Among patients with suspected myocardial ischemia the metabolic syndrome is gradually and independently predictive for future cardiovascular events in men and women, and in patients with diabetes mellitus type 2 as well as in non-diabetic patients. Insulin resistance does not explain the full amount of risk inferred by the metabolic syndrome.

THE PORTFOLIO DIET: COMBINATION OF PLANT FOODS WITH KNOWN HYPOCHOLESTEROLEMIC PROPERTIES TO REDUCE SERUM LIPIDS

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Although drugs and diet have both been shown to be effective in reducing cardiovascular disease risk and mortality, the apparent ineffectiveness of current dietary strategies to reduce serum cholesterol by comparison with statins has reduced enthusiasm for diet as a therapeutic option. To increase the effectiveness of diet in reducing serum cholesterol, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (11) and the American Heart Association (12) recently recommended the use of functional foods or foods high in components which reduce cholesterol as an additional dietary strategy. These functional ingredients include viscous fibers, soy protein, plant sterols and nuts, all of which are permitted by the US Food and Drug Administration to carry a health claim that they reduce the risk of cardiovascular disease. Individually they are well recognized to lower serum cholesterol by 4%-7%. In combination on metabolic diets, cholesterol reductions approaching lovastatin, a first generation statin, have been reported. In self selected (ad libitum) diets, mean 13% LDL cholesterol reductions can be sustained over a 6 month period, with just under one third of the subjects achieving LDL-C reductions of approximately 70% of those achieved with the initial dose of first generation statins. Application of diet, for those serious about attempting dietary change, may achieve clinically meaningful reductions in lipid risk factors for CHD.

DETERMINANTS OF THE CONVERSION OF DIETARY ALPHA LINOLENIC ACID INTO EPA AND DHA IN HUMANS

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Fish oil fatty acids such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are believed to prevent cardiac death and other diseases. The human body can convert alpha-linolenic acid (ALA, C18:3n-3) from plants into EPA and DHA, but the efficiency of this conversion is controversial. We used compartmental modelling to estimate the *in vivo* conversion of ALA into EPA, docosapentaenoic acid (DPA C22:5n-3) and DHA. During 28 days, twenty-nine healthy adults followed a diet providing about 7 percent of energy from linoleic acid (C18:2n-6) and 0.4 % from ALA. On day 19, subjects received a single bolus of 30 mg ^{13}C-ALA and for the next eight days 10 mg twice daily. To estimate hepatic conversion of n-3 fatty acids a tracer model was developed based on the averaged ^{13}C-data of the participants. A similar tracee model for unlabeled ALA was obtained using the averaged ^{12}C values, the kinetic parameters derived from the tracer model, and mean ALA consumption. We found that that nearly 7% of dietary ALA was incorporated into plasma phospholipids. From this pool, 99.8% was converted into EPA, and 1 % into DPA and subsequently into DHA.

Thus the conversion of alpha-linolenic acid into longer-chain polyunsaturates is limited. In addition it is uncertain whether internally produced fatty acids have the same health effects as fatty acids taken by mouth. Therefore the final test of the efficacy of alpha-linolenic acid in the prevention of cardiac death should be a clinical trial with hard end points. Such a trial is under way in the Netherlands.

THE OMEGA-3 INDEX: A NEW RISK FACTOR FOR SUDDEN CARDIAC DEATH?

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Low intakes or blood levels of eicosapentaenoic and docosahexaenoic acids (EPA+DHA) are independently associated with increased risk of death from coronary heart disease (CHD). In randomised secondary prevention trials, fish or fish oil have been demonstrated to reduce total and CHD mortality at intakes of about 1 g/d. Red blood cell (RBC) fatty acid composition reflects long-term intake of EPA+DHA, and is a surrogate for myocardial omega-3 FA content. The RBC EPA+DHA (hereafter called the Omega-3 Index) has many of the characteristics of a risk factor for death from CHD. We conducted clinical and laboratory experiments in order to generate data necessary for the validation of the Omega-3 Index as a CHD risk predictor. The relationship between this putative marker and risk for CHD death, especially sudden cardiac death (SCD), was then evaluated in several, published primary and secondary prevention studies. The Omega-3 Index was inversely associated with risk for CHD mortality. An Omega-3 Index of approximately 8% (EPA+DHA as a percent of total RBC FA) was associated with the greatest cardioprotection, whereas an Index of less than 4% was associated with the least. Published data from the Physicians' Health Study suggests that the Omega-3 Index may be a more powerful predictor of risk for SCD than classic CHD risk factors. We suggest that an Omega-3 Index of 8% or greater be considered as a preliminary cardioprotective target value. The Omega-3 Index appears to be a novel, physiologically-relevant, easily- and safely-modified, independent and graded risk factor for death from CHD that could have significant clinical utility.

CORRELATIONS BETWEEN BLOOD FATTY ACIDS AND DIETARY HABITS, PHYSIOLOGICAL CONDITIONS, LIFE STYLES AND FATTY ACID SUPPLEMENTATIONS, ASSESSED THROUGH AN INNOVATIVE ANALYTICAL APPROACH

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Dietary fatty acids (FA), e.g. the intakes of polyunsaturated fatty acids (PUFA), in particular the n-3 PUFAs, are important in the maintenance of the health status. The assessment of the FA status in large populations groups is however limited by the complexity and costs of conventional methods for blood collection, sample preparation and analysis. A new approach (Marangoni et al. Anal.Biochem. 2004), for the analysis of FA in a drop of blood collected from a fingertip, was applied to screen 100 volunteers (54 M vs 46 F, 22-73 y), and questionnaires for food consumption were also used. The following differences were observed : Gender : higher Linoleic Acid (LA) and lower Oleic Acid (OA) in F vs M. Dietary habits : higher Arachidonic Acid in high vs low meat consumers, higher levels of n-3 FA (especially DHA) in high vs low fish consumers, higher LA in high vs low vegetable consumers. Life styles: lower n-3 FA in smokers vs non smokers. Physiological conditions: lower PUFA in pregnant women. FA intakes: both the consumption of salmon (200 g/week) and the intake of 1capsule /day of n-3 FA for two weeks resulted in distinct elevations of blood EPA and DHA levels. The new method has the following advantages : rapid, simple, less expensive, no requirement of health personnel, applicable to population groups, provides information on the impact of dietary habits, life styles and FA supplementation on blood FA. It will be very useful in epidemiological studies, in screening subjects/patients for dietay interventions, and in studies aimed at establishing correlations between pathological states and blood FA.

NOT THE a-LINOLENIC TO LINOLEIC ACID RATIO, BUT THE AMOUNTS OF DIETARY a-LINOLENIC AND LINOLEIC ACID, DETERMINE *IN VIVO* CONVERSION OF a-LINOLENIC ACID

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This study was designed to examine if the *in vivo* conversion of ALA is influenced by the dietary ALA:LA ratio or by the absolute amounts of ALA and LA (ALA: a-linolenic acid, LA: linoleic acid). For this, 29 subjects received for 4 weeks a control diet (7 En% LA, 0.4 En% ALA, ALA:LA of 1:19). For the next 6 weeks, 9 subjects received the control diet, 10 subjects a low-LA diet (3 En% LA, 0.4 En% ALA), and 10 subjects a high-ALA diet (7 En% LA, 1.1 En% ALA). The ALA to LA ratio was 1:7 in both experimental diets. Ten days before the end of both periods, subjects received daily a small dose of uniformly labelled ^{13}C-ALA. Based on fasting plasma phospholipid concentrations of ^{13}C- and ^{12}C-labeled n-3 fatty acids, *in vivo* n-3 conversion was quantified using compartmental modelling. Incorporation of ALA into plasma phospholipids (as % of ALA-intake) changed from 7.1% to 8.4% on the control diet, from 6.3% to 11.5 % ($p=0.012$) on the low-LA diet, and from 9.7% to 3.1% on the high-ALA diet ($p<0.001$). In absolute amounts, ALA incorporation rose from 72.0 to 120.3 mg ($p=0.020$) on the low-LA diet, while it remained unaffected on the high-ALA diet. On all diets, >99% of ALA from the phospholipid pool was converted into EPA. The relative conversion of EPA into DPA and DHA did not change on the experimental diets but - in absolute terms - it increased from 0.7 to 1.9 mg ($p=0.001$) on the high-ALA diet.

In conclusion, the absolute amounts of ALA and LA in the diet - rather than their ratio - determine *in vivo* ALA conversion.

AN INTEGRATIVE METABOLISM APPROACH IDENTIFIES STEAROYL-COA DESATURASE AS A TARGET FOR AN ARACHIDONATE-ENRICHED DIET.

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Epidemiological studies have correlated diets containing higher intakes of PUFA with lower rates of chronic diseases (such as coronary heart disease, obesity, *etc.*). Using an integrative metabolism approach to explore the molecular mechanisms regulated by the chronic consumption of PUFA, the liver and hippocampal transcriptome and lipid-metabolome of mice fed a control diet, an arachidonate-enriched fungal oil, a docosahexaenoic acid-enriched fish oil, or a combination of the two oils were examined. Significant differences in the lipid-metabolomic profile were identified by singular value decomposition analysis. Hepatic gene transcription and fatty acid (FA) metabolism were significantly altered by diets enriched with arachidonate. The total levels and FA composition of several phospholipid (PL) species were significantly changed, with phosphatidylcholine (PC) demonstrating the greatest alterations. Reduced PC levels were linked to decreased expression of critical enzymes in PC biosynthesis (choline kinase, -2.2 fold; glycerol-3-phosphate acyltransferase, -2.0 fold). Alterations in PL-FA composition were related to decreased expression of FA biosynthetic genes (fatty acid synthetase, –3.7 fold; stearoyl-CoA desaturase-1 (SCD1), -1.8 fold). Furthermore, hepatic SCD1 expression levels were reflected in FA metabolism through increased concentrations of palmitic (fungal oil, +45%; combination, +106%) and stearic acids (fungal oil, +60%; combination, +63%) in PC. The robust analysis of the lipid-metabolome, reinforced with gene expression data, suggests that the chronic feeding of an arachidonate-enriched diet modulates molecular endpoints similar to those previously reported in the obesity-resistant SCD1-/- mouse, namely, genes involved in lipid oxidation/synthesis and the significant changes in FA metabolism stemming from a repressed SCD1 activity.

DIETARY CONTRIBUTION TO CARDIOVASCULAR RISK FACTORS IN SUBJECTS WITH CARDIOVASCULAR DISEASE IN AUSTRALIA AND NEW ZEALAND

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In the secondary cardiovascular prevention trial, LIPID, in which patients treated with pravstatin experienced significantly fewer cardiovascular events, a subgroup of 1077 patients was surveyed at intervals to determine their pattern of nutrient consumption. Objectives were to establish whether food consumption differed between Australians and New Zealanders and whether regular counselling improved compliance and whether initial eating habits influenced clinical outcomes after 5 years. The characteristics of the subgroup and total cohort were similar.
New Zealanders, who within the whole trial of 9014 patients suffered significantly more cardiovascular events, were found in this subgroup to have eaten significantly more energy from total, saturated and monounsaturated fat at entry, reflected in a higher concentration of LDL cholesterol. Patients with previous coronary artery bypass grafting had altered their diets more appropriately than other categories of patients with CHD. Surprisingly, obese and diabetic patients consumed more fat, saturated fat and cholesterol than lean and non-diabetic patients.
Relationships between nutrients and plasma lipids confirmed significant direct effects of saturated fatty acids on LDL cholesterol and of alcohol on plasma triglyceride and HDL cholesterol. Glycemic load related inversely with HDL cholesterol. Regular dietary counselling led to significant improvement in compliance with guidelines that might have obscured the possibility of finding relationships between consumption patterns at entry and further cardiovascular events after 5 years.
The main finding is that the difference in cardiovascular events and mortality between two populations with similar socio-economic and cultural backgrounds might be partly explained by differences in the amounts and type of fats eaten that appears to have been reflected also in the differences in LDL cholesterol concentrations.

MODERATE WEIGHT-LOSS BUT NOT FISH OILS AFFECT FASTING AND POSTPRANDIAL MARKERS FOR ENDOTHELIAL ACTIVATION AND INFLAMMATION

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Obese and overweight persons are at risk for cardiovascular diseases, which may relate to their pro-inflammatory serum profiles. Indeed, weight-loss lowers CHD risk, but weight maintenance is difficult. Therefore, dietary approaches - such as fish oils - that improve inflammatory serum profiles are of relevance. In this study we therefore evaluated n-3 fatty acids vs moderate weight loss side-by-side, both fasting and during the postprandial phase. 11 normolipidemic healthy men (BMI 30-35 kg/m^2) participated in a double blind trial in which each subject received in random order 1.35 g/d fish oils or oleic acid (control) as part of a recommended diet for 6 weeks. In the 3rd period, 8 out of 11 subjects consumed low caloric diets (2MJ/day) followed by a period of weight stabilization. At the final day of all 3 periods, a postprandial test was conducted. Weight-loss lowered both fasting and the postprandial TG response (P<0.001), while fish oil improved only postprandial TG (P=0.006). Fasting CRP was lowered by weight-loss (P=0.027, 95% CI – 1.54 to –0.13 mg/l) but not by fish-oil. Since CRP did not change postprandial, the lowered fasting CRP levels were maintained throughout the postprandial phase (P<0.001). Fish-oil did not affect s-ICAM while weight loss significantly reduced fasting (P=0.009, 95% CI –25.6 to –5.3 pg/ml), and the postprandial s-ICAM response (P<0.001). Fasting s-ICAM and TG correlated (r=0.68; P=0.029), as did changes in fasting s-ICAM and TG both during weight-loss (r=0.80; P=0.029) and fish-oil (r=0.76; P=0.009). In addition a cytokine antibody array approach was evaluated as inflammatory signature. These findings indicate that, 1.35 g/d fish oils supplied for 6 weeks - in contrast to 10 kg weight-loss - do not improve endothelial dysfunction and inflammatory characteristics associated with CHD. This implies that possible cardioprotective effects of fish oil cannot be explained via effects on inflammation.

MACROPHAGE EXPRESSION OF ACTIVE MMP-9 INDUCES PLAQUE RUPTURE IN APOE-/- MICE

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It is becoming increasingly clear that the majority of the clinical consequences of atherosclerosis are due to the physical rupture of advanced atherosclerotic plaques. It has been hypothesised that macrophages play a key role in inducing plaque rupture by secreting proteases that destroy the extracellular matrix components that provide physical strength to the fibrous cap. Despite many reports detailing the expression of multiple proteases by macrophages in rupture-prone shoulder regions, there is no direct proof that macrophage-mediated matrix degradation can induce plaque rupture. We set out to test this hypothesis by using a novel macrophage-specific retroviral vector to overexpress the candidate enzyme MMP-9 in macrophages of advanced atherosclerotic lesions of ApoE-/- mice. Despite a greater than 10-fold increase in the expression of MMP-9 by macrophages, there was only a minor increase in the incidence of plaque rupture. Subsequent analysis revealed that macrophages secrete MMP-9 predominantly as a pro-form, and this form of the enzyme is unable to degrade the matrix component elastin. Expression of an auto-activating form of MMP-9 in macrophages in vitro greatly enhances elastin degradation, and induces significant plaque rupture when overexpressed by macrophages in advanced atherosclerotic lesions of ApoE-/- mice in vivo. These data show for the first time that enhanced macrophage proteolytic activity can induce plaque rupture, and highlight MMP-9, and factors that regulate its activation, as potential therapeutic targets for stabilising rupture-prone plaques.