

**2nd INTERNATIONAL
SYMPOSIUM ON
TRIGLYCERIDES AND HDL:
ROLE IN CARDIOVASCULAR
DISEASE AND THE
METABOLIC SYNDROME**

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HDL AND TRIGLYCERIDES: AN EVOLVING STORY IN CARDIOVASCULAR PREVENTION

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In contrast with elevated low-density lipoprotein cholesterol (LDL-C), the role of low high-density lipoprotein cholesterol (HDL-C) and hypertriglyceridemia (HTG) in cardiovascular prevention has garnered less attention. Epidemiologic studies have made a case for low HDL-C as independent coronary heart disease (CHD) risk factor while HTG remains controversial. The presence of all three lipid risk factors (i.e., the Lipid Triad) may help discriminate a higher risk subgroup of patients from the general population who may warrant more intensive therapy. Low HDL-C and HTG are common to the dyslipidemias present in diabetes or the metabolic syndrome. Identifying strategies for the management of these abnormalities and determining whether treatment goals are needed are important considerations for future discussions. The utilization of low HDL-C and HTG is congruent with the concept of global risk assessment, in which the intensity of treatment is based on a patient's overall risk for developing CHD. This presentation will discuss the history of these two risk factors while noting points of intersection and divergence in this evolving narrative.

ATHEROSCLEROSIS AND THE METABOLIC SYNDROME: UNDERSTANDING BASIC MECHANISMS AND NEW TARGETS

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Multiple mechanisms may contribute to the increased incidence of cardiovascular events, and hence atherosclerosis, found in the metabolic syndrome. As is the case with diabetes mellitus, some of these mechanisms appear unique to the metabolic syndrome and a pre-diabetic state while others are common to both diabetic and non-diabetic atherosclerosis. One unifying element to the atherosclerosis linked to the various components of the metabolic syndrome may be inflammation. This pro-inflammatory state may be an additional target for therapeutic intervention. In this regard, peroxisome proliferator-activated receptors (PPARs) have received attention as a mechanistic player and therapeutic target in the metabolic syndrome and its cardiovascular complications. PPAR- γ activation, with drugs like thiazolidinediones, decreases insulin resistance, a postulated key element in the metabolic syndrome. PPAR- α activation, by fibrates, improves the dyslipidemia found in the metabolic syndrome. Considerable evidence suggests PPAR agonists may limit inflammation and atherosclerosis, which may broaden their potential therapeutic use in conditions like diabetes and the metabolic syndrome. Likewise, the ongoing development of dual PPAR- α/γ agonists may have particular relevance for the metabolic syndrome. An alternate interpretation to this body of evidence would suggest that perhaps diabetes, dyslipidemia the metabolic syndrome derive from some deficit in endogenous PPAR ligand(s) production. We have demonstrated that lipoprotein lipase, a key enzyme in triglyceride metabolism, can generate PPAR ligands and activate PPAR pathways, even recapitulating the anti-inflammatory effects of these agonists. Additional studies support the presence of a network of lipase-lipoprotein network that regulates nuclear receptor responses and may provide links between the metabolic syndrome and vascular responses.

METABOLIC SYNDROME: EPIDEMIOLOGICAL AND GENETIC OVERVIEW

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The metabolic syndrome is a multi-dimensional risk factor for atherosclerotic cardiovascular disease (ASCVD) and type 2 diabetes. The syndrome consists of five metabolic risk factors: atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, a prothrombotic state, and a proinflammatory state. The syndrome also is multi-factorial in origin. Among the latter factors are both environmental and genetic components. The environmental components include obesity and physical inactivity. Genetic factors constitute various disorders of adipose tissue, primary insulin resistance, and genetic dysregulation of each of the five risk factors. There is considerable ethnic variability in susceptibility for the metabolic syndrome. For example, South Asians represent a population that has a high prevalence of primary insulin resistance. Persons from this population commonly develop metabolic syndrome and its complications with only moderate obesity. On the other hand, in the United States, the prevalence of the syndrome is high because of a high prevalence of marked obesity even when the genetic contribution is relatively small. In addition, there is ethnic variation in the pattern of the syndrome. Thus Asians and Native Americans are more likely to develop type 2 diabetes than other patterns when they become obese. Populations of African origin tend to show hypertension as the first manifestation of the metabolic syndrome, whereas Caucasians usually exhibit atherogenic dyslipidemia first. In spite of these general trends, there is considerable individual variability within each population or ethnic group in their particular expressions of the syndrome. In spite of this variability in expression, the metabolic syndrome as a whole is becoming increasingly important in the causation of ASCVD and type 2 diabetes in all populations around the world.

METABOLIC SYNDROME AND CVD: OVERVIEW OF EPIDEMIOLOGY AND CLINICAL TRIALS TO REDUCE ATHEROSCLEROSIS

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The metabolic syndrome (MS) carries an increase in the risk of coronary disease (CAD) and diabetes (DM). There is general agreement that insulin resistance is a feature of the MS. In populations, fasting levels of serum insulin correlate with resistance to insulin. Fasting hyperinsulinemia and increased CAD risk, at least in men, has been recognized for some years. Recently, direct measures of insulin sensitivity have supported these suggestions. Even in DM, where the increase in CAD risk is well recognized, some suggest that this is seen only in those who are resistant to insulin. There are many factors in insulin resistant people that may contribute to their increased CAD risk. Most intervention studies have focused on correcting lipoprotein abnormalities, reducing blood pressure, and altering life style. There are no studies of CAD risk reduction specifically in the MS. The rationale for intervention is based on extrapolation. The lipoprotein abnormalities in the MS are: increased triglyceride, reduced HDL-cholesterol, normal LDL-cholesterol, and small dense LDL particles. Both in those with and those without DM, reducing LDL-cholesterol results in a reduction of CAD risk. While there is general agreement that "the lower, the better" for LDL-cholesterol in those without DM, there have been no studies of target levels for LDL-cholesterol specifically in DM or the MS. The effect of treating with fibrates on CAD risk has been studied in those with and without DM. These drug reduce triglyceride, increase HDL-cholesterol and shift populations of LDL to larger more buoyant particles. These changes are accompanied by a reduction in CAD events and a reduction in the progress of angiographically evaluated CAD, particularly in those with MS. Some need both a fibrate and a statin for all of their lipoprotein abnormalities. Many fear that this will increase the risk rhabdomyolysis. That is true if the fibrate is gemfibrozil, but it does not apply to fenofibrate, particularly if the combination is used with appropriate caution and in individuals who are unlikely to develop muscle problems.

ASSESSMENT AND MANAGEMENT OF PATIENTS WITH METABOLIC SYNDROME AND ABDOMINAL OBESITY

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The metabolic syndrome consists of a constellation of metabolic risk factors for atherosclerotic cardiovascular disease. These include atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, a prothrombotic state, and a proinflammatory state. Since all of these metabolic risk factors are not identified in routine clinical practice, the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) recommended a simple method for making a diagnosis of the metabolic syndrome in clinical practice. Thus, if a patient has any three of the following five features, a diagnosis of the syndrome can be made: waist circumference > 102 cm in men or >88 cm in women, fasting triglycerides \geq 150 mg/dL, HDL cholesterol < 40 mg/dL in men or < 50 mg/dL in women, HDL cholesterol < 40 mg/dL in men or < 50 mg/dL in women, blood pressure \geq 130 mmHg systolic or \geq 85 mmHg diastolic, and fasting glucose \geq 100 mg/dL. In some populations, a lower waist circumference can count as being elevated, e.g. \geq 90 cm in men or \geq 80 cm in women in Asian populations. Recently the International Diabetes Federation (IDF) proposed a similar clinical definition of metabolic syndrome except that increased waist circumference as a requirement for diagnosis. Patients who are found to have the metabolic syndrome by either ATP III or IDF criteria are at increased long-term risk for developing both clinical atherosclerotic disease and type 2 diabetes. For this reason, they should enter long-term clinical management to reduce their risk. First-line management consists of lifestyle therapies including weight reduction and increased physical activity. For those who are at elevated short-term risk for cardiovascular disease or diabetes, drug therapy may be required to reduce the metabolic risk factors. In these patients, drugs may be necessary for reducing LDL-cholesterol levels, mitigating atherogenic dyslipidemia, lowering blood pressure, and controlling elevated glucose (if type 2 diabetes is already present). In addition, low-dose aspirin therapy may be beneficial for patients at higher risk.

THE RIO PROGRAM: IMPROVEMENT OF METABOLIC PARAMETERS AND NEW DATA FROM RIO DIABETES

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Although physicians have pharmacological tools to treat common metabolic complications, there are currently no available treatments targeting the most common cause of these clustering abnormalities: abdominal obesity. The Endocannabinoid (EC) System has been shown to play a key role in the regulation of energy balance, and blockade of this system could have a significant improvement in fat distribution and lipid/glucose metabolism. The RIO-Lipids, RIO-Europe and RIO-North America trials are studies of the Phase III RIO programme, conducted in more than 6,600 overweight/obese subjects, investigating the efficacy of rimonabant, the first selective CB1-blocker, on metabolic risk factors.

Endpoints for these 3 trials included metabolic parameters such as HDL-cholesterol, triglycerides (TG) and insulin sensitivity, as well as waist circumference and metabolic syndrome. The pooled data from these three trials provides a large patient sample and highlights the robustness of the results replicated throughout the trial.

All three trials showed a significant reduction in waist circumference and weight after 1 year of treatment with rimonabant 20 mg. There was also a significant improvement -partly independent from weight loss- in metabolic parameters, including increased HDL, reduced TG and improved insulin sensitivity. These improvements led to a significant decrease in the number of patients criteria with the ATP III metabolic syndrome. RIO-Europe and North America 2-year results show that chronic therapy with rimonabant maintains the efficacy for weight and metabolic improvements achieved during the first year

Data on diabetes metabolic control, waist changes and metabolic aspects will be presented from the recent described RIO Diabetes Trial. The results from the four RIO studies highlight the significant improvement in metabolic parameters, achieved with rimonabant 20 mg, and show an important role in the reduction of multiple cardiovascular risk factors.

WAIST AND TG: SCREENING TOOLS TO DETECT PATIENTS WITH INCREASED ABDOMINAL ADIPOSITY

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The metabolic syndrome (MS) is being increasingly recognized as an important risk factor for cardiovascular disease (CVD). Clinical features of the MS include a preferential accumulation of adipose tissue in the abdominal area, insulin resistance, hyperglycemia and compensatory hyperinsulinemia, moderated to marked hypertriglyceridemia, reduced plasma HDL cholesterol levels and hypertension. Other metabolic abnormalities that have been associated with the MS are small dense LDL particles, increased LDL particle number (hyperapoB), impaired postprandial lipemia, impaired fibrinolytic activity and subclinical inflammation. The National Cholesterol Education Program, Adult Treatment Panel III (NCEP-ATP III) has recently proposed a definition to help clinicians diagnose patients with the MS. Their proposed diagnostic is based on the presence of at least 3 of the following five features: 1- waist girth > 102 cm in men and > 88 cm in women, 2- plasma TG levels > 1.7 mmol/l, 3- plasma HDL-C levels < 1.0 in men and < 1.3 in women, 4- blood pressure > 135/85 mmHg, and 5- plasma glucose levels > 6.1 mg/dl. We have recently proposed a simplified approach based on only two measurements, i.e. an increased waist circumference and moderately elevated plasma triglyceride levels. There is accumulating data indicating that characterizing this hypertriglyceridemic waist phenotype may prove to be effective in identifying high risk populations with a preferential accumulation of abdominal fat and with the MS. Recent data identified the hypertriglyceridemic waist phenotype as the best indicator of cardiovascular risk in postmenopausal women. Other organizations such as the WHO have also proposed definitions for the MS that differ to various extent from the one proposed by the NCEP. Although each definition has its own subtleties, all are based on a common rationale, which is the presence of a cluster of metabolic abnormalities related to an insulin resistance state and/or abdominal obesity.

EMERGING ROLE OF HDL-C: IS THE TIME RIGHT FOR HDL-C GUIDELINES?

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Epidemiologic studies have made a case for low high-density lipoprotein cholesterol (HDL-C) as an independent coronary heart disease (CHD) risk factor. Low HDL-C is often present among the dyslipidemias that accompany diabetes or the metabolic syndrome. Identifying strategies for the management of this lipid abnormality is an important consideration for future discussions. While current guidelines specify levels of HDL-C that determine risk, guidelines have not universally endorsed a target level of HDL-C for treatment. This presentation will examine what role, if any, HDL-C should play as a target of therapy, considering the current landscape of available therapies.

ROLE OF HDL AND ITS SUBFRACTIONS IN CARDIOVASCULAR DISEASE RISK REDUCTION

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Decreased HDL-C (< 40 mg/dl or 1 mmol/L) has been shown to be an independent CHD risk factor. Raising HDL C with resins, fibrates, statins, and most notably with niacin, has been associated with significant benefit in CHD risk reduction. Fibrates and statins will modestly raise HDL C, while niacin causes significant increases, especially in combination with a statin (30% increases in HDL C can be achieved). Associated with these increases are dramatic increases in large alpha 1 HDL particles, which predict CHD in the Framingham Offspring Study and angiographic progression or regression in coronary atherosclerosis in the HATS Trial. These particles interact very efficiently with SRB1-the liver receptor important for reverse cholesterol transport. Infusing ApoA-I-Milano-phospholipid has also been shown to promote regression. The combination of a statin and a cholesterol ester transfer protein (CETP) inhibitor is currently under intense study in this regard. CETP inhibition is very effective in raising large alpha 1 HDL particles. The sequence of HDL metabolism is: 1). production of apoA-I in liver and intestine with the formation of very small pre-beta HDL 2). efflux of cholesteryl ester and phospholipids from cells via ABCA1 to pre-beta HDL to form small discoidal alpha 4 HDL 3). esterification of free cholesterol on alpha 4 HDL by lecithin:cholesterol acyl transferase (LCAT) to form spherical large alpha 1 HDL and smaller alpha 2 and 3 particles containing both apoA-I and apoA-II 4). transfer of cholesteryl ester from alpha 1 and 2 HDL to liver via SRB1 or 5). to triglyceride rich lipoproteins of intestinal or liver origin in exchange for triglyceride via the action of CETP to form alpha 3 HDL particles; 6) the removal of triglyceride and phospholipids from alpha 3 HDL via the action of lipoprotein, hepatic, and endothelial lipases to form small pre-beta HDL and alpha 4 HDL, and the repetition of the cholesterol efflux cycle.

EVIDENCE TO SUPPORT AGGRESSIVE MANAGEMENT OF HDL-C: IMPLICATIONS OF RECENT TRIALS

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Although statins are shown to be effective in reducing by approximately 30% the risk of coronary heart disease morbidity and mortality, expanded approaches are needed to extend these results. Many patients at risk for coronary disease have low concentrations of HDL cholesterol, for which the risk is additive to that of LDL. Treatment strategies for low HDL cholesterol incorporate lifestyle changes including exercise, but the effect of these interventions on HDL-C levels is modest. Niacin is the most effective, currently available drug to increase HDL-C, with typical dose-dependent increases of 20-25% possible. Niacin has been shown to prevent coronary events as monotherapy, as shown in Coronary Drug Project, and in combination therapy with simvastatin, as shown in the HDL-Atherosclerosis Treatment Study. However, the incremental benefit of adding niacin to statin therapy has been uncertain. The publication of the ARBITER-2 trial has now provided an important, preliminary answer to this question. In this randomized, double-blind, placebo controlled trial, niacin (extended release niacin, Niaspan, 1gm/d) was added to existing statin therapy in individuals with known coronary heart disease with well-controlled LDL-C. After 1 year, the addition of niacin was shown to significantly reduce the progression of carotid intima-media thickness by 68%. Increases in HDL were directly related to more favorable changes in carotid intima-media thickness. Although needing confirmation in clinical events trials, this is the first study to show the incremental benefit of a strategy of combination statin-niacin therapy.

LIPOPROTEIN SUBCLASSES, INFLAMMATION, AND CARDIOVASCULAR RISK IN THE METABOLIC SYNDROME

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Low levels of high-density lipoprotein cholesterol (HDL-C) are common among patients with early-onset cardiovascular disease (CVD), and most often, these findings are not isolated. Low HDL-C is a component of the metabolic syndrome in 35.2% of US adult men and 39.3% of adult women, and is usually associated with other lipoprotein abnormalities. Subjects with low HDL-C levels have small cholesterol-depleted HDL and low-density lipoprotein (LDL) particles. Small LDL particles have an increased susceptibility to oxidative modification. Oxidized LDL contributes to the development of atherosclerotic lesions in several ways, including accumulation in macrophages to form foam cells, and modulation of various pro-inflammatory pathways. HDL particles may protect against atherosclerosis by providing anti-oxidant protection to LDL particles by scavenging reactive oxygen species and inhibiting activation of nuclear factor- κ B signaling cascade. Data from the Framingham Offspring Study demonstrated that subjects whose HDL-C was <1.0 mmol/L (39 mg/dL) had considerably higher LDL particle numbers than indicated by their LDL-cholesterol level because of the excess of small cholesterol-depleted LDL particles. High numbers of LDL particles identify individuals at greatest risk for atherosclerotic vascular disease and cardiovascular events. In the Women's Health Study, low HDL-C levels improved the strength of association between high LDL particle number and major cardiovascular events. As high levels of LDL particles are a robust predictor of cardiovascular events, strategies targeted at raising low levels of HDL-C need to account for HDL-LDL particle interactions.

COMBINATION THERAPY FOR MULTIPLE LIPID DISORDERS: A GLIMPSE INTO THE FUTURE

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Although clinical trials of statin therapy have demonstrated large reductions in low-density lipoprotein cholesterol (LDL-C) and statistically significant reductions in risk for coronary artery disease (CAD) events in both primary and secondary prevention, many patients with CAD have lipid abnormalities besides elevated LDL-C. Combining a statin, which inhibits cholesterol synthesis, with agents with complementary mechanisms may provide greater improvements for the entire lipid profile and greater benefits clinically for atherothrombotic disease. In patients with mixed dyslipidemia, combination therapy with niacin, a fibrate, or another agent may be required to normalize high-density lipoprotein cholesterol (HDL-C) and triglyceride-rich lipoproteins. Combination therapy can provide greater reductions in LDL-C and triglyceride and greater increases in HDL-C than statin monotherapy. Large randomized trials are being conducted to evaluate incremental additional clinical benefit of combination therapy regimens versus statin monotherapy in different patient populations.

MECHANISMS BY WHICH COMPONENTS OF THE METABOLIC SYNDROME MIGHT IMPACT THE ARTERY WALL

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The major components of the metabolic syndrome are dyslipidemia (hypertriglyceridemia, low HDL and the presence of small dense LDL), hypertension, dysglycemia, central obesity and inflammation. The mechanisms by which the metabolic syndrome leads to atherosclerosis are unknown, but likely involve interaction of some of these risk factors with the artery wall. Dyslipidemia, hypertension and hyperglycemia all can lead to endothelial dysfunction. Small dense LDL binds to and is retained with increased avidity by vascular proteoglycans, and also demonstrates increased oxidation susceptibility. Low HDL levels might lead to impaired reverse cholesterol transport. Hypertension and hyperglycemia are associated with increased oxidative stress. In addition to increasing the oxidative modification of lipoproteins, oxidative stress may play a role in facilitating retention of atherogenesis lipoproteins by vascular proteoglycans. Inflammation may also play an important role in atherogenesis in the metabolic syndrome. Central obesity and the metabolic syndrome are associated with an increase in inflammatory molecules such as C-reactive protein and serum amyloid A. Both these molecules can interact directly with the artery wall and potentially influence atherogenesis by a variety of mechanisms, including an increased tendency to thrombosis. Thus many components of the metabolic syndrome can interact with the artery wall to potentially explain the accelerated atherosclerosis seen in this syndrome.

DECIPHERING THE PHENOTYPIC AND GENETIC COMPLEXITY OF METABOLIC SYNDROME

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The goal of the GEMS cohort, a large multi-centre family study, is to gain insight into the molecular basis of Metabolic Syndrome and atherogenic dyslipidemia (ADL), defined as low HDL and high triglyceride. A total of 3273 subjects (504 families) have been recruited in this study. Overall, a good concordance was observed between the GEMS definition and ATPIII. To better understand the phenotypic structure of ADL, and in attempt to identify clusters of variables that may represent a common biological pathway or origin, we applied cluster analysis to a set of 14 biomarkers or anthropometric measures. In addition, we used variance components analysis to estimate heritability. A four cluster solution was generated which explained 65% of the variance. The clusters obtained included a body fat component, two lipid clusters and a blood pressure component. All components were significantly heritable ($H^2:0.3-0.6$). A whole genome scan was performed on the families. Categorical analysis on ADL revealed peaks with $LOD>3$ on chromosomes 4, 11 and 14. In addition, we performed QTL analyses using triglyceride, HDL, LDL size and the cluster scores as variables. Here again a number of significant peaks ($LOD>3$) were identified on chromosomes 8 and 15 whereas suggestive peaks ($LOD>2$) were found on chromosomes 1, 2, 5, 6, 9, 10, 11, 16. A total of 25 candidate genes which are known to influence plasma lipid levels have also been genotyped and will be integrated into the linkage analysis.

INSULIN RESISTANCE: LESSONS FROM HUMAN LIPODYSTROPHIES

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Human lipodystrophies represent a group of diseases characterized by altered body fat repartition and major metabolic alterations with insulin resistance leading to increased cardio-vascular risk. Studies on animal and human models of lipodystrophies outlined the importance of adipose tissue in metabolism and in the production of numerous cytokines and hormones collectively termed adipokines. Human lipodystrophies are generally characterized by a reduction of peripheral fat and/or an increase in visceral fat. These modifications could result in insulin resistance and altered glucose and lipid levels through mechanisms involving increased free fatty acid release together with altered production of adipokines such as TNF-alpha, IL-6 and adiponectin. These alterations could lead to increased lipid content in muscles and liver and insulin resistance. The role of local cortisol production in visceral fat hypertrophy is discussed.

Genetic forms of lipodystrophies are rare and regroup mainly 3 groups of diseases. Congenital generalized lipodystrophy or Berardinelli-Seip syndrome, recessive, is characterized by a complete early lipatrophy and severe insulin resistance and results from mutations in either the seipin or the AGPAT2 gene encoding an enzyme involved in triglyceride synthesis. Familial partial lipodystrophies are dominant and include the Dunnigan syndrome due to mutations in the gene encoding lamin A/C, belonging to the complex group of laminopathies, and forms linked to mutations in the PPAR-gamma gene resembling the metabolic syndrome. The metabolic syndrome represents the most common form of lipodystrophy. HIV-infected patients often present lipodystrophies, mainly related to antiretroviral drugs side effects, together with insulin resistance and metabolic alterations.

Such syndromes help to understand the mechanisms involved in insulin resistance resulting from altered fat repartition and could benefit from treatment with insulin sensitizers.

RELATIONSHIPS BETWEEN CHOLESTERYL ESTER TRANSFER ACTIVITY AND CORONARY RISK FACTORS IN METABOLIC SYNDROME

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Increased levels of small-dense low-density lipoprotein (SD-LDL) are a risk factor for coronary heart disease (CHD). Low HDL-C and increased triglycerides are features of the metabolic syndrome linked in many patients by cholesteryl ester transfer protein (CETP) activity, but the relationship between CETP activity, SD-LDL and other risk factors is less well established. We determined SD-LDL apo B by ultracentrifugation and immunoassay in 70 men aged >40 years with (n = 37) and without (n = 33) metabolic syndrome. CETP activity was determined using an isotopic method with quantification of CE transfer between endogenous HDL and apo B-containing lipoproteins. Compared with patients without metabolic syndrome, patients with metabolic syndrome had a higher SD-LDL apo B (18 [4-58] vs. 12 [3-44] mg/dl; $p<0.001$), CETP activity (23 [7-56] vs. 14 [6-76] nmol/ml/h; $p<0.005$), CRP (2.54 [0.13-9.08] vs. 0.70 [0.09-3.75] mg/l; $p<0.001$) and lower adiponectin concentrations (1.84 [0.70-4.60] vs. 2.28 [0.71-7.53] mg/l; $p<0.02$). Using results for all patients, CETP activity correlated with SD-LDL apo B ($r = 0.542$; $p<0.001$) and with triglycerides ($r = 0.751$; $p<0.001$). Both CETP activity and SD-LDL apo B correlated inversely with HDL-C ($r = -0.386$; $p<0.005$ and $r = -0.375$; $p<0.005$, respectively). In patients with metabolic syndrome alone, serum paraoxonase activity correlated inversely both with triglycerides ($r = -0.474$, $p<0.005$) and CRP ($r = -0.299$, $p<0.05$) and serum apo B ($r = -0.411$, $p<0.005$). Serum adiponectin correlated directly with HDL-C both in patients with- and without metabolic syndrome ($r = 0.330$; $p<0.05$ and $r = 0.303$; $p<0.05$, respectively). In addition to drugs lowering triglycerides these findings suggest that treatment of metabolic syndrome with CETP inhibitors might offer particular benefit.

HIGH TRIGLYCERIDES, LOW HDL CHOLESTEROL, AND SMALL LDL PARTICLES PREDICT INCIDENT TYPE 2 DIABETES IN NON-DIABETIC CORONARY PATIENTS

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Hypothesis: Patients with type 2 diabetes mellitus (T2DM) exhibit a typical pattern of dyslipidemia with high triglycerides, low HDL cholesterol, and small LDL particles, which is also frequently observed in pre-diabetic patients. We therefore hypothesized that diabetic dyslipidemia is predictive of incident T2DM among non-diabetic individuals. **Methods:** We enrolled 362 non-diabetic patients undergoing coronary angiography for the evaluation of CAD. Diabetes was diagnosed according to ADA criteria; thus at baseline all patients had a fasting plasma glucose (FPG) ≤ 125 mg/dl and no patient was receiving antidiabetic medication. The incidence of T2DM was recorded after a follow-up period of 4 years. **Results:** From our non-diabetic coronary patients, 172 had normal fasting glucose (NFG) < 100 mg/dl, and 190 had impaired fasting glucose (IFG) ≥ 100 mg/dl at baseline. After 4 years, T2DM was newly diagnosed in 15 patients. IFG was associated with a strongly increased risk of T2DM (adjusted HR = 5.56 [1.23-25.14]; $p = 0.026$). Whereas serum values of total cholesterol, LDL cholesterol, and apolipoprotein B were not associated with the incidence of T2DM, triglycerides (standardized adjusted HR = 2.12 [1.35-3.34]; $p = 0.001$), and, inversely, HDL cholesterol (HR = 0.32 [0.12-0.80]; $p = 0.015$) as well as the LDL particle diameter (HR = 0.06 [0.01-0.49]; $p = 0.008$) proved significantly predictive of incident T2DM in our cohort of coronary patients. **Conclusions:** From our data we conclude that among patients undergoing coronary angiography IFG is strongly predictive of incident T2DM. Importantly, high triglycerides, low HDL cholesterol, and a small LDL particle diameter also are significant predictors of the 4-year incidence of T2DM among non-diabetic coronary patients.

UNUSUAL PATHWAYS OF LIPID UPTAKE BY MUSCLES

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Mice with overexpression or tissue specific knockout of LPL were used to dissect the role(s) of LPL in lipid uptake, plasma lipoprotein metabolism, and muscle function. Loss of LPL only in the heart led to hypertriglyceridemia and increased postprandial lipemia. LPL deficient hearts compensated by increasing basal glucose uptake. Surprisingly, these mice developed cardiac dysfunction characterized by decreased fractional shortening, and interstitial and perivascular fibrosis.

Mice with transgenic expression of anchored LPL (LPL^{GPI}) on cardiomyocytes develop dilated cardiomyopathy. When the LPL^{GPI} transgene was bred onto the knockout of endogenous heart LPL, cardiac dysfunction deteriorated further. This suggested that excess fatty acid uptake was not responsible for the cardiomyopathy in LPL^{GPI} mice. Rather, it appeared that these animals had a cholesterol induced muscle dysfunction. LDL uptake into these hearts was double that of control mice. However, deletion of LDL receptors by crossing onto the LDL receptor knockout background did not alter LDL uptake. It also did not affect LDL uptake into skeletal muscles. LDL uptake into the quadriceps muscle was increased by statin treatment. In mice that also had excess LPL expression in skeletal muscle, statin treatment led to increased cholesterol-content and elevation of plasma CPK. Thus, in this model augmentation of LDL uptake is associated with statin-induced skeletal muscle dysfunction.

EASILY ASSESSABLE CLINICAL PREDICTORS OF INSULIN RESISTANCE: GEMS AND ADOPT STUDY

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Background: Insulin resistance (IR) is a feature in the natural history of type 2 diabetes (T2D) and potentially of cardiovascular disease. Methods of determining IR are frequently invasive or require fasting insulin concentrations. The aim was to identify easily assessable clinical parameters that can reliably predict IR as determined by the homeostatic model assessment (HOMA-S) in two different populations: Genetic Epidemiology of Metabolic Syndrome (GEMS) & A Diabetes Outcome Prevention Trial (ADOPT). GEMS is a family-based study designed to explore the genetic basis of metabolic syndrome. GEMS consist of 3273 individuals from 504 families comprised of affected (defined by low HDL and high triglyceride levels in plasma) & non-affected. ADOPT consists of drug naïve population with T2D patients.

Methods: The baseline clinical measures included were age, sex, race, BP, TG, HDL, ratio of TG: HDL, waist-hip ratio, waist and hip circumference from both studies. Additionally adiponectin, Apo B, CRP, leptin were also available for the GEMS study and urinary albumin: creatinine ratio for the ADOPT study (n=4092). Adjusting for all these measures, the topmost variable of importance was obtained by Random Forest Method and adjusted means by using mixed effects model.

Results: In both studies TG:HDL ratios(-0.2, $p < 0.0001$) and BMI(-0.5, $p < 0.0001$) correlated significantly with IR after adjusting for other clinical measures. Leptin(-0.3, $p < 0.0001$) was also another important predictor in the GEMS study. The age-sex-BMI adjusted means of HOMA-%S were 111.1, 102.7, 82.5, 68.1 (in GEMS) and 41.7, 34.8, 30.9, 25.1 (in ADOPT) for the 4 quartiles of TG:HDL ratio ($p < 0.001$).

Conclusions: Among the clinical measures that are easily assessable, the best predictors of IR were TG:HDL ratio and BMI in two different populations.

UPDATE ON ENDOTHELIAL LIPASE

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Endothelial lipase (EL) is part of a lipase subfamily that includes lipoprotein lipase (LPL) and hepatic lipase (HL). Many of the typical features of this subfamily are conserved in EL, but its activity is much more toward a preference for phospholipids as a lipid substrate and for HDL as a lipoprotein substrate compared with LPL and HL. Structure-function studies indicate important roles for multiple regions of the EL molecule in determining its lipid and lipoprotein substrate preferences. EL is markedly upregulated by inflammatory stimuli *in vitro* and *in vivo*. EL is expressed not only in endothelial cells but in macrophages as well. Overexpression and loss-of-function studies in mice indicate that EL plays an important role in HDL metabolism and atherosclerosis. Genetic studies and studies of plasma EL mass are consistent with a role of EL in HDL metabolism and atherosclerosis in humans. Thus, EL may be an important factor in regulating HDL metabolism and influencing atherosclerosis in humans.

ENDOTHELIAL LIPASE: RECOMBINANT EXPRESSION, PURIFICATION AND HTS ASSAY DEVELOPMENT

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Endothelial lipase (EL) plays a key role in HDL hydrolysis, therefore pharmacological inhibition of EL might have the potential to raise plasma HDL in humans and inhibit atherosclerotic process.

We cloned human EL gene (*LIPG*) from placenta cDNA and we expressed it as recombinant protein (rEL) in insect cells. The expression conditions were optimized to obtain an efficient secretion of the enzyme in a catalytically active form. The scouting of different chromatographic approaches defined the purification strategy to recover a partially purified protein with fully preserved catalytic properties. This procedure led to a final yield of 0.2 mg of rEL/liter of insect cells.

A homogeneous, fluorescence-based assay was designed in a miniaturized 384-MTP format using as substrates fluorogenic surrogate phospholipids and triglycerides: the purified enzyme displayed high phospholipase A1 activity and very low triglyceride lipase activity, in agreement with literature data. The assay conditions (e.g., reaction temperature, pH, buffer, salts, artificial membranes, tolerance to DMSO) were optimized to maximize the phospholipase A1 activity of rEL and the corresponding kinetic parameters (K_m , V_{max} , turnover number, specificity constant) were determined. In the final configuration, the assay uses K_m concentration of fluorogenic substrate (5 μ M) and 3 nM of rEL. Under these conditions, the signal-to-background ratio in the linear range of the reaction is about 3 and the standard deviation is < 5%. The adaptability of the assay to 1536 MTP format was also proved by end-point measurement, with an almost comparable performance in comparison with the 384-MTP format. Reproducible dose-dependent response of the reaction to reference inhibitors was demonstrated, with IC_{50} values in the low nanomolar range.

In conclusion, we believe that the availability of an assay for EL suitable for high-throughput screening may greatly contribute to the discovery of novel chemical entities with a potential for therapeutic intervention in the atherosclerotic cardiovascular disease.

CLINICAL SIGNIFICANCE OF MEASURING LIPOPROTEIN LIPASE (LPL) MASS IN PREHEPARIN PLASMA FOR THE DIAGNOSIS OF LPL ABNORMALITY

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Objective: We aimed to clarify a clinical significance of measuring LPL mass in plasma before the injection of heparin (preheparin plasma) for the diagnosis of LPL abnormalities in comparison with LPL mass in plasma after the injection of heparin (postheparin plasma: PHP).

Subjects and Methods: Obligate homozygotes (n=12) and heterozygotes (n=39) for LPL mutations, identified from Japanese, and healthy subjects (n=41) were studied. Preheparin plasma and PHP (30 units/kg of body weight) were obtained from the subjects after 12 hrs fasting. LPL mass was measured by a MARKIT-M LPL ELISA kit (Dainippon Pharmaceutical Co., Ltd) using two distinct monoclonal antibodies to human LPL molecule, which was capable of measuring LPL mass concentration of 0 to 300 ng/ml without dilution of plasma.

Results: LPL mass values [mean (SD)] in PHP were 6.4(9.8), 100.4(33.1) and 227.1(61.7) ng/ml for homozygotes, heterozygotes and healthy subjects, while those values in preheparin plasma were 2.3(5.0), 26.0(13.9) and 51.4(20.4) ng/ml. As previously reported, three groups (homozygotes, heterozygotes and healthy subjects) were clearly distinguished by LPL mass values in PHP, whereas LPL mass values in preheparin plasma overlapped between heterozygotes and healthy subjects. However, ROC (receiver operating characteristic curve) analysis indicated a diagnostic significance for the measurement of LPL mass in preheparin plasma by using cut-off-point of 30.2 ng/ml, i.e., diagnostic sensitivity and specificity were 82.4% and 87.8%, respectively. **Conclusions:** LPL mass measurement in preheparin plasma can be used for the diagnosis of LPL abnormality as the first screening.

NOVEL ETHNIC-SPECIFIC ASSOCIATIONS BETWEEN ENDOTHELIAL AND HEPATIC LIPASE POLYMORPHISMS AND NMR HDL SUBCLASSES AND SIZE

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Background: Men of African origin have consistently been found to have a more favorable, less atherogenic lipoprotein profile than Caucasian men, characterized by lower levels of triglycerides and higher levels of HDL-C. However, the presence of atherosclerosis is more strongly associated with HDL particle size distribution than with HDL-C level. Small HDL is thought to be atherogenic and large HDL cardio-protective. We hypothesized that polymorphisms 584C>T in the endothelial lipase (*LIPG*), and -514C>T in the hepatic lipase (*LIPC*) genes play a significant role in determining the less atherogenic lipoprotein profile. **Methods:** Lipoproteins were measured by NMR spectroscopy in Caucasian (N=600) and African-American (N=100) men older than 65 from the Cardiovascular Health Study, and in Afro-Caribbean men (n=205) older than 65 from the Tobago Health Study. **Results:** The frequency of the *LIPG* 584T allele was 6% in African-Caribbeans, 14% in African-Americans and 29% in Caucasians. In Afro-Caribbeans, the *LIPG* 584T allele was associated with less small HDL and greater HDL size, whereas in Caucasians and African-Americans, no significant association was found. We suspect that this may be due to a lower genetic heterogeneity or presence of gene-environmental interaction in Afro-Caribbeans. In contrast, the frequency of the *LIPC* -514T allele was much higher in Afro-Caribbeans (57%) and in African-Americans (49%) than in Caucasians (20%). The *LIPC* -514T allele in both populations of African origin, but not in Caucasians, was associated with elevated large HDL and greater HDL size. **Conclusions:** The current analyses reveal novel ethnic-specific genetic effects on HDL subclass and size phenotypes. Our findings may have important implications for the understanding of ethnic differences in lipoprotein distributions and susceptibility to atherosclerosis.

ENDOTHELIAL LIPASE AND LIPOPROTEIN METABOLISM IN HUMAN

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Little is currently known on the contribution of Endothelial lipase (EL) to variations in *in vivo* lipoprotein kinetics in humans. The present study was therefore undertaken to investigate how EL correlates with variations in intravascular apoB and apoA containing lipoprotein kinetics. *In vivo* kinetic studies were performed in a sample of 18 healthy men (mean age [\pm SD] 42.1 \pm 9.5 yrs, body mass index 29.8 \pm 4.6 kg/m²). Plasma EL concentrations were measured by ELISA using a polyclonal antibody and the *in vivo* kinetics of apoA-I, apoA-II (d < 1.25 g/l), VLDL-apoB, IDL-apoB and LDL-apoB were studied using a primed-constant infusion of l-(5,5,5-D³)-leucine for 12 h under feeding conditions. Kinetic parameters were generated by multicompartmental modelling of the enrichment data over time with SAAM II. VLDL-apoB fractional catabolic rate (FCR) correlated negatively with plasma EL levels ($r=-0.48$; $P=0.05$). Subjects were divided into two EL groups based on the 50th percentile of plasma EL levels (above or below 1738 ng/mL). Men in the high EL group were characterized by a lower VLDL-apoB FCR (6.45 \pm 1.42 pools/day) compared to men in the low EL group (10.20 \pm 1.54 pools/day, $P=0.03$). ApoA-I production rate tended to correlate negatively with plasma EL levels among these men ($r=-0.43$; $P=0.076$), suggesting that higher plasma EL levels were associated with a reduced production of apoA-I. These results suggest that EL may be implicated in modulating the *in vivo* metabolism of apoB-containing lipoproteins and HDL in human. These results also further reinforce previous observations, which have suggested that elevated plasma EL levels are associated with a deteriorated lipoprotein-lipid profile.

DIETARY COMPOSITION VERSUS CALORIC RESTRICTION FOR THE TREATMENT OF METABOLIC SYNDROME

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There is a rising prevalence of obesity in our society, which in turn is related to an increased prevalence of various CHD risk factors. Visceral adiposity has been linked to elevated free fatty acids, insulin resistance, and in turn to increased type 2 diabetes, decreased thrombolysis, hypertriglyceridemia, decreased HDL, small dense LDL, and increased levels of inflammatory markers including CRP. The metabolic syndrome has been defined by ATP III as having 3 or more of the following traits: waist > 40 inches (102 cm) in men and > 35 inches (88 cm) in women, HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women, blood pressure > 130/85 mmHg, fasting triglyceride > 150 mg/dl, and fasting glucose > 110 mg/dl. Meeting these criteria has been associated with a 3.5 fold increased risk of CHD compared to controls. There is consensus that decreasing caloric intake and increasing physical activity is the treatment of choice in these subjects, along with pharmacologic therapy if indicated. There is also consensus that diets for CHD prevention should be low in saturated fat (< 7% of calories) and cholesterol (< 200 mg/day). However there is debate about the level and type of dietary carbohydrate, fat, and protein. We have tested the popular diets: Atkins, Ornish, Weight Watchers, and Zone, and have found that all these diet plans can promote weight loss and CHD risk reduction, with self-rated compliance to any plan being the best predictor of weight loss rather than the particular diet used. Moreover the carbohydrate restricted diets have the benefit of promoting more triglyceride lowering and HDL raising, while the fat restricted diets are more effective for LDL lowering. Our own recent controlled feeding trials clearly indicate that dietary glycemic index (GI) is important in regulating plasma insulin levels in the obese, especially in the fed state, and therefore the GI of the diet may be important for maintaining long-term weight loss.

COMPARISON OF THE EFFECTS OF POPULAR DIETS ON METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE.

Frederick F. Samaha

Obesity is associated with increased risk of cardiovascular events, largely through its association with multiple factors associated with diabetes and metabolic syndrome, including elevated triglyceride levels, depressed HDL-C levels, high cholesterol, hypertension, and a pro-inflammatory state. This presentation will provide an overview of studies evaluating the impact of several popular diets on cardiovascular risk.

Studies of low fat diets (<30% total fat) have generally shown a decrease in total cholesterol and LDL-C, but also a reduction of HDL-C, with no favorable effect on triglycerides. One such trial showed a reduction in myocardial infarction after 16 years, but this study was confounded by several simultaneous interventions.

Extreme low fat diets ($\leq 15\%$ total fat) have been evaluated in a small number of studies. One, the Lifestyle Heart Trial, found a 28% reduction in total cholesterol, but a 10% drop in HDL-C, and a 13% increase in triglycerides. Angiography did show a decrease in CAD progression, although these findings were possibly confounded by other interventions.

Four recent studies compared a low carbohydrate/high fat diet to a low fat diet. All 4 studies found greater weight loss at 6 months on the low carbohydrate diet, but the two that extended follow up to 1 year found no difference. Cholesterol failed to decrease with the low-carbohydrate diet, but there were more favorable effects on HDL-C and triglycerides, and better glycemic control in patients with diabetes.

Mediterranean diets, in part, focus on substituting unsaturated fats for saturated fats. Studies of these diets have shown modest weight loss, a reduction in total cholesterol and triglycerides, and also an increase in HDL-C. Importantly, outcomes studies have demonstrated a striking reduction in cardiovascular events, possibly related to either favorable lipid effects or antiarrhythmic effects of n-3 polyunsaturated fats.

In summary, these studies lend evidence to an evolving consensus that focusing on the quality of fats (i.e. substituting unsaturated fats for saturated fats), and reigning in the over-consumption of simple carbohydrates, will have a favorable impact on cardiovascular disease.

WHAT IS THE BEST DIET FOR TREATING THE METABOLIC SYNDROME?

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Altering dietary habits is the cornerstone of weight loss therapy for obese patients. Although many different diets have been shown to induce short-term weight loss, poor long-term compliance and weight regain are common. The optimum diet for effective, safe, and lasting weight loss is unknown. Several intrinsic factors within food may be involved in regulating energy intake. These factors include macronutrient composition, energy density, fiber content, fat and sugar substitutes, portion size, food variety, and the physical properties of food (eg, taste and feel). In addition, extrinsic factors related to societal, social, and economic influences, including food cost, availability, variety, and marketing also affect food intake. Therefore, effective diet therapy may need to consider both intrinsic and extrinsic food factors to achieve successful long-term weight loss in obese patients.

VERY LOW-CARBOHYDRATE DIETS ARE SUPERIOR TO LOW-FAT DIETS FOR TREATING LIPID DISORDERS IN SUBJECTS WITH ELEVATED TRIGLYCERIDES AND LOW HDL CHOLESTEROL

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Very low-carbohydrate (VLC) diets significantly decrease triglycerides (TG) and increase HDL-C, whereas low-fat (LF) diets have the opposite effect. As part of an ongoing study, we are comparing the effects of a VLC (<10% carbohydrate) versus a LF (<30% fat) diet in overweight men and women with high TG (>150 mg/dL) and low HDL-C (men <40 mg/dL, women <50 mg/dL). Fasting blood collections were performed on two separate days for determination of lipids and an oral fat tolerance test was done before and after the diet to assess postprandial lipemia. Currently 15 out of 40 subjects have completed the 12 wk intervention ($n = 15$, age 37.6 ± 13.0 yr, body mass 98.3 ± 13.7 kg, BMI 33.8 ± 4.8 kg/m²). Serum total cholesterol, LDL-C, HDL-C, and TG were not significantly different in subjects randomized to the VLC diet ($n = 9$; 213 ± 31 , 136 ± 24 , 34 ± 5 , and 217 ± 52 mg/dL, respectively) and the LF diet ($n = 6$; 201 ± 35 , 125 ± 35 , 39 ± 6 , and 184 ± 74 mg/dL, respectively). There were no significant differences between diets for the change in total cholesterol (VLC -4%, LF -5%) and LDL-C (VLC 6%, LF -3%). There were significant interaction (diet x time) effects for fasting HDL-C and TG and postprandial lipemia. HDL-C was significantly increased after the VLC diet (16%) and unaffected by the LF diet (0%). Fasting TG were significantly decreased after the VLC (-57%) and LF (-11%) diet. Postprandial lipemia area under the curve was significantly decreased after the VLC diet (-55%) but not the LF diet (9%). These preliminary results indicate that VLC diets have consistent and dramatic favorable effects on the lipid disorders associated with metabolic syndrome.

DIETARY LONG CHAIN MONOUNSATURATED FATTY ACIDS (LC-MUFA) AND HEXACOSANOIC ACID (C26:0) IN RED BLOOD CELLS, A NEW FACTOR FOR ATHEROSCLEROSIS

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Our colleagues have shown that an elevated level (%) of hexacosanoic acid (C26:0), a very long chain saturated fatty acid (VLCFA), among the total fatty acids in red blood cells (RBC) is significantly correlated with atherosclerotic risk factors such as age, total cholesterol (T-cho), TG, HDL-C, LDL-C, obesity and smoking, as well as with hypertension and diabetes mellitus (Antoku et al. *Atheroscler* 2000;153:169-173). These results raise the possibility that C26:0 might be a cause of such lifestyle-related diseases. VLCFAs (C>20) are degraded by peroxisomal beta-oxidation and are deposited in the tissues of patients with peroxisomal disorders, leading to multiple organ dysfunction. It is well established that Lorenzo's oil containing long chain monounsaturated fatty acids (LC-MUFAs) can reduce the plasma C26:0 level in these patients. We hypothesized that certain types of fish oil containing LC-MUFA, such as cod liver oil, might be effective to prevent atherosclerosis.

A human study showed that intake of 5g of cod liver oil for 11 weeks significantly reduced the RBC C26:0 and LDL-C levels, and also significantly increased HDL-C. Also, oral administration of cod liver oil (1g/kg body weight) to LDL receptor-deficient rabbits (WHHL rabbit) significantly reduced T-cho, TG, and LDL-C, while it significantly increased HDL-C.

From these results, fish oil containing high levels of LC-MUFAs could be a possible form of preventive nutrition against atherosclerosis. Further investigations are needed to determine the mechanisms by which C26:0 is related to the metabolic syndrome.

C-REACTIVE PROTEIN LEVELS PREDICT THE LIPID AND LIPOPROTEIN RESPONSE TO DIETARY INTERVENTIONS.

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Studies have yet to compare the impact of a low fat diet and a high monounsaturated fatty (MUFA) acid diet, under unrestricted energy intake conditions, on plasma C-reactive protein (CRP) levels. In order to investigate this issue, 61 men (mean age 37.5 ± 11.5 (SD) years, mean body mass index 29.0 ± 5.0 kg/m²) were randomly assigned to a moderately low fat (25.8% of energy intake from fat) diet or to a high fat diet rich in MUFA (40.17% of energy intake from fat, 22.5% from MUFA) consumed under *ad libitum* conditions for a period of 6-7 weeks. All meals were provided to study participants over the course of the study. Plasma CRP levels were measured using a highly sensitive assay. Diet-induced changes in plasma CRP levels in response to the low fat diet and the high MUFA diet were not statistically significant and the effects of the two experimental diets on plasma CRP levels were also comparable. However, baseline CRP levels predicted the lipoprotein-lipid responsiveness to the experimental diets. After the low fat diet, plasma and VLDL-triglycerides (TG) levels were significantly reduced in the subgroup with low CRP levels at baseline (P<0.05 for both), but increased in the subgroup with high CRP levels (P=0.08 and P<0.05, respectively). The high MUFA diet-induced reductions in plasma TG, VLDL-TG and VLDL-cholesterol levels were significant only within the subgroup with low CRP at baseline (P<0.0001). These results suggest that while a low fat diet and a high MUFA diet may have no significant effect on mean plasma CRP levels, baseline CRP appear to modulate the diet-induced changes in lipid and lipoprotein levels. Specifically, our data indicated that individuals with low plasma CRP concentrations were more likely to show a beneficial reduction in TG levels in response to dietary changes than individuals with high plasma CRP levels at baseline.

OMEGA - 3 SUPPLEMENTATION ENHANCES BODY FAT LOSS DURING EXERCISE

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Both regular exercise and inclusion of fish and/or fish oil rich in omega-3 (ω3) fatty acids in the diet can improve cardiovascular (CV) health. We examined whether the combination of both may be a more effective strategy in reducing CV and metabolic risk than either treatment alone.

Sixty-five overweight subjects (mean BMI=34) were assigned to one of four groups; fish oil (FO), FO and exercise (FOX), sunflower oil (SO, control), SO and exercise (SOX) in a double-blind dietary intervention trial. The FOX and FO groups consumed 6 g/day of HiDHA® tuna oil (~1.6g DHA; NuMega Ingredients), while the SOX and SO groups took 6 g/day of sunflower oil for 12 weeks. The FOX and SOX groups walked for 45 min, 3 days/wk at 75% of their age-predicted maximal heart rate. Plasma triglycerides and respiratory exchange ratio (RER) during exercise were assessed at 0, 6 and 12 weeks. Body composition was assessed by Dual Energy X-ray Absorptiometry (DEXA) at 0 and 12 weeks.

Significant changes in body composition were evident in the FOX group only; this group lost significantly more body fat than any of the other groups (p < 0.05). This improvement was associated with an increase in fat oxidation during exercise (p < 0.01), as indicated by a reduction in the respiratory exchange ratio (RER). Plasma triglyceride concentrations were reduced significantly in the FO and FOX groups compared with the SO and SOX groups, indicating that this effect was independent of exercise.

Fish oil and exercise appear to have a synergistic effect on reduction of body fat, suggesting that ω3 supplementation may form a useful adjunct to exercise programs aimed at improving body composition and CV risk.

METABOLIC CONSEQUENCES OF THERAPEUTIC WEIGHT REDUCTION

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It is more difficult to sustain weight reduction than to achieve it. There is potent physiological opposition to the maintenance of a reduced body weight. Evolutionarily, such a system for defending body fat would function in service of survival and the maintenance of reproductive integrity during periods of scarcity. We have found that maintenance of a reduced weight by lean or overweight subjects provokes decreased 24 hour energy expenditure that is 300-500 kcal/day less than that predicted solely on the basis of weight lost. This decline in energy expenditure is mainly due decreased energy expended in physical activity reflecting an approximately 15-20% increased skeletal muscle work efficiency. In addition, decreased sympathetic nervous system tone, circulating concentrations of leptin and bioactive thyroid hormones, and increased parasympathetic nervous system tone and circulating concentrations of cortisol act coordinately to favor rapid regain of lost weight. These adaptations to weight loss are similar to the phenotypes of leptin-deficient humans and rodents. Leptin administration to weight-reduced subjects reverses most of these metabolic adaptations. The normalization of these systems following leptin administration suggests that the weight-reduced state may be regarded as a condition of relative leptin insufficiency.

DECREASED ADIPONECTIN LEVELS IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA (FCH) PREDICT THE ATHEROGENIC LIPID PROFILE

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Objective: FCH is characterized by elevated levels of plasma total cholesterol, triglycerides and apolipoprotein B. Other features of FCH are obesity and insulin resistance. Low levels of adiponectin are associated with insulin resistance and accelerated atherogenesis. We determined whether decreased adiponectin levels contribute to the phenotypes of FCH.

Methods The study population comprised 158 patients, 390 normolipidemic (NL) relatives and 89 spouses. FCH diagnosis was based on apoB, TG and TC levels using the nomogram (Vleuten et al Circulation 2004;109:2980-5). Serum adiponectin levels were determined by ELISA.

Results For both males and females, the mean adiponectin level ($\mu\text{g/ml}$) was significantly lower in FCH patients (2.0 (1.8-2.2) and 2.5 (2.3-2.8), respectively) compared to NL relatives (2.3 (2.2-2.5) and 3.1 (2.8-3.3), respectively), and spouses (2.4 (2.1-2.7) and 3.2 (2.8-3.6), respectively). These differences remained after adjusting adiponectin levels for waist circumference and insulin resistance. Compared to patients with high adiponectin levels (3rd tertile), FCH patients with low adiponectin levels (1st tertile) were more obese (waist (cm) 92.2 (88.8-95.5) versus 83.0 (79.7-86.3)), more insulin resistant (HOMA 3.7 (3.2-4.3) versus 2.3 (2.0-2.7)), had higher TG levels (mmol/l) (3.2 (2.8-3.6) versus 2.3 (2.1-2.6)), low HDLc levels (mmol/l) (0.89 (0.82-0.96) versus 1.06 (0.98-1.13)) and more small, dense LDL (K-value -0.37 (-0.44 — -0.30) versus -0.15 (-0.22 — -0.08) ($K < 0.1 = \text{sdLDL}$)). Independent of waist circumference and IR, a low adiponectin level in FCH patients was the strongest predictor of the athero-genic lipid profile (TG $\beta = -0.219$, HDL $\beta = 0.222$, K-value $\beta = 0.329$).

Conclusion Low adiponectin levels contribute to the atherogenic lipid profile in FCH independent of obesity and insulin resistance. This may imply a role of adipose tissue metabolism in the pathophysiology of FCH.

ADIPOCYTE FATTY ACID BINDING PROTEIN (AP2), A NEWLY IDENTIFIED LXR TARGET GENE, IS INDUCED BY LXR AGONISTS IN HUMAN THP-1 CELLS.

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The Liver X receptors (LXR α and LXR β), ligand-activated transcription factors, belong to the superfamily of nuclear hormone receptors and have been shown to play a major role in atherosclerosis by modulating cholesterol and triglyceride metabolism. In this report, we describe a novel LXR target, the adipocyte fatty acid binding protein (aP2), which plays an important role in fatty acid metabolism, adipocyte differentiation as well as atherosclerosis. Exposure of human monocytes and macrophages (undifferentiated as well as differentiated THP-1 cells) to LXR agonists (natural or synthetic) results in a time- and concentration-dependent increase in aP2 mRNA levels. In addition, the increase in aP2 mRNA level was additive when the cells were treated with LXR and PPAR γ agonists. Also, RXR agonist induced aP2 expression in these cells. While no additive effect was observed when THP-1 cells were treated with LXR and RXR agonists simultaneously, additive as well as synergistic effects were observed when differentiated and undifferentiated THP-1 cells were treated with RXR and PPAR γ agonists. In contrast, LXR agonists have no effect on aP2 expression in human adipocytes. Analysis of the human aP2 promoter revealed a potential LXR response element (LXRE). Gel shift data showed that the LXR α /RXR α heterodimer bound to the LXRE motif in aP2 promoter *in vitro* in a sequence-specific manner. Deletion and mutation analyses of the proximal aP2 promoter firmly demonstrate this is a functional LXRE. These data indicate that human aP2 promoter is a direct target for the regulation by LXR/RXR heterodimers. Through its regulation in macrophages, aP2 may represent a new therapeutic target for the prevention of atherosclerosis.

ADIPOSE TISSUE REGULATES PLASMA LIPID LEVELS AND ADIPOSITY VIA FASTING-INDUCED ADIPOSE FACTOR/ ANGIOPOIETIN-LIKE PROTEIN 4

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- Proteins secreted from adipose tissue play an important role in the regulation of glucose metabolism. However, much less is known about their effect on lipid metabolism. The Fasting-Induced Adipose Factor (FIAF, ANGPTL4, PGAR, HFARP) is a protein secreted from adipose tissue, expression of which is under control of PPAR α , PPAR β/δ and PPAR γ in a variety of tissues. To further elucidate the function of FIAF, transgenic mice were generated that over-express FIAF in white adipose tissue (WAT). Transgenic animals had 50% less WAT compared to their wild-type littermates, although food intake was equal. In FIAF-Tg mice plasma levels of triglycerides and (HDL)-cholesterol were significantly elevated. Plasma lipid profiles showed that the increase in triglycerides was attributable to an elevated VLDL fraction, and was most likely due to inhibition of lipoprotein lipase, suggesting that FIAF impairs fat deposition. Interestingly, FIAF was present specifically in the HDL-cholesterol fraction, both in human and mouse. In human plasma, FIAF concentration was positively correlated with HDL-cholesterol levels and was increased by treatment with the PPAR α agonist fenofibrate. Overall, our data indicate that FIAF promotes leanness by preventing fat deposition and is an important determinant of plasma lipid levels. Based on these data it can be hypothesized that disturbances in FIAF signalling are involved in dyslipidemia.

ASEX-SPECIFIC DETERMINANTS OF ADIPONECTIN IN OLDER ADULTS: THE ROLE OF SERUM SEX HORMONES

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Low levels of the adipocyte-derived hormone adiponectin have been linked to insulin resistance, type 2 diabetes, and atherosclerotic heart disease. Circulating adiponectin levels are higher in women than men; the biological basis for this sex difference is unknown. We examined the sex-specific association of adiponectin with multiple factors thought to affect its levels including age, body size (BMI), fat distribution (waist girth), current smoking, alcohol intake, physical activity, insulin resistance (HOMA-IR) and endogenous sex hormone levels in 1510 older men and women (mean age=72.3) from the Rancho Bernardo Study. Analyses were adjusted for potential confounding effects of HDL cholesterol and triglycerides. Median serum adiponectin was 50% lower ($P < 0.001$) in men (9.8 mg/L) than women (15.5 mg/L). In unadjusted analyses, adiponectin was positively related to age, alcohol intake, HDL, and testosterone, and negatively related to waist girth, BMI, HOMA-IR, triglycerides and bioavailable estradiol in both men and women (all $P < 0.01$). Adiponectin did not differ in current smokers or in those who exercised 3 or more times per week. Sex-specific multivariate linear regressions adjusting for HDL and triglycerides showed that only a few factors (age, HOMA-IR, and sex hormones) were independently associated with circulating adiponectin, and these factors were the same for men and women. The most striking finding was that higher levels of endogenous testosterone and lower bioavailable estradiol concentrations each predicted higher adiponectin. This was true for both men and women, and was not explained by differences in age, adiposity, alcohol intake, insulin resistance, or lipoprotein levels. These sex hormone associations are opposite to those that could explain sex differences in adiponectin. Thus, sex differences in circulating adiponectin levels in older adults are not dependent on sex hormone regulation. Adiponectin regulation by the factors studied here is similar for men and women.

OBESITY, PLASMA HIGH SENSITIVITY C-REACTIVE PROTEIN LEVELS AND INSULIN RESISTANCE STATUS AMONG SCHOOL CHILDREN IN TAIWAN

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To evaluate the degree of obesity and plasma high sensitivity C-reactive protein (hs-CRP) levels in relation to insulin resistance status among school children in Taiwan. After multistage sampling, we randomly selected 1439 children (702 boys and 739 girls) with the mean age of 13.5 years (from 12 to 16) in Taipei in 2003. Anthropometric measures, blood pressure (BP), and plasma biochemical variables (including lipid profiles, glucose and insulin) were measured using standard methods. Plasma hs-CRP levels were measured using nephelometric methods. We calculated insulin resistance (IR) index using HOMA methods and further calculated a gender-specific insulin resistance symptom (IRS) summary score by adding the quartile ranks from the distribution of systolic BP, triglyceride (TG), HDL-C and insulin levels of each children. A high IRS summary score corresponds to higher levels of SBP, TG and insulin levels and lower levels of HDL-C. In general, boys were tall, heavier, had larger BMI and higher BP than girls; however, there is no difference on plasma hs-CRP, IR index and IRS summary score between genders. Plasma hs-CRP levels were positively correlated with anthropometric measures, diastolic BP, TG and insulin levels and IR index and IRS summary score. Anthropometric measures, IR index and IRS summary score were significantly higher in children with plasma hs-CRP levels greater than 3.0 mg/L when compared with < 1.0 mg/L (test for trend $p < 0.05$). However, plasma hs-CRP levels were not associated with plasma insulin levels, IR index and IRS summary score after adjusting for BMI in both genders. Plasma hs-CRP levels were positively correlated with anthropometric measures and the components of IRS (such as BP, lipid profiles and glucose levels) among school children. However, the degree of body fat status (as measured by BMI) play a more significantly role on insulin resistance status than hs-CRP among Taiwanese children.

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INCREASING HDL AS AN EMERGING APPROACH TO THE TREATMENT OF CVD

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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. Current approaches to increasing HDL to protect against premature CVD include both acute and chronic HDL therapy. Acute HDL therapy with 5 infusions of apoA-I Milano/phospholipids complexes resulted in a regression of total atheroma vol by 4.2% in 36 patients compared to 11 controls following an acute coronary event utilizing intravascular ultrasound to quantitate coronary atheroma (Nissen et al. JAMA 2003;290:2292-2300). This dramatic reduction in atherosclerosis in 6 weeks provided a major milestone in acute HDL therapy. It is anticipated that acute HDL therapy with infusions with selectively delipidated HDL or apoA-I mimetic peptides will result in a similar reduction in atherosclerosis. An additional peptide approach is the oral administration of D-4F, a peptide which is being developed as an anti-atherosclerotic agent based primarily on its anti-inflammatory properties. The most promising approach to chronic therapy to raising HDL is the inc. in HDL associated with the administration of a CETP inhibitor under development by both Pfizer and Roche. Initial Phase 1 studies with CETP inhibition with Torcetrapib in man were associated with no change in total plasma cholesterol (10-120mg/bid) but an inc. in HDL-C from 16 to 91% (10-120mg/bid) and a dec. in LDL-C from 7% to 42% (60mg/d-120mg/bid) (Clark et al. Arter.Thromb.Vasc.Biol. 2004;24:490-497). N.Eng.J.of Med. 2004;350:1505-1515). These combined results provide support for the concept that raising HDL both as acute as well as chronic therapy may represent a new therapeutic approach for the treatment of CVD. Future clinical trials will provide critical information on the efficacy of these new approaches to increase HDL and decrease atherosclerosis.

ROLE OF HIGH DENSITY LIPOPROTEINS IN COMBATING VASCULAR INFLAMMATION.

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The best-known anti-atherogenic property of HDLs relates to their ability to promote the efflux of cholesterol from cells in the artery wall. However, HDLs may also protect by virtue of antioxidant, anti-thrombotic and anti-inflammatory properties. We have demonstrated that HDLs inhibit both the cytokine-induced and the CRP-induced expression of adhesion proteins in endothelial cells growing in cell culture. We have also shown in studies conducted in vivo in normocholesterolemic rabbits that the acute inflammatory response induced in carotid arteries by implantation of non-occlusive periarterial collars is virtually abolished by infusion of reconstituted HDLs (rHDL) consisting of discoidal complexes of apoA-I and phospholipids or by the infusion of lipid-free apoA-I alone. When rabbits are infused only with saline, insertion of the carotid collar induces a marked infiltration of neutrophils into the artery wall and generation of substantial amounts of reactive oxygen species by the artery. However, if the animals are infused intravenously with rHDLs or lipid-free apoA-I prior to, at the time of or up to three hours after of insertion of the periarterial collar, both the infiltration of neutrophils and the generation of reactive oxygen species are essentially abolished. The precise mechanism underlying these anti-inflammatory effects of HDLs and lipid-free apoA-I is uncertain. Despite this, the therapeutic implications are substantial, providing strong support for a proposition that the infusion of rHDL or lipid-free apoA-I should be investigated as potential first-line therapy to minimize tissue damage in states of acute vascular inflammation such as acute coronary syndromes, stroke and ischemia-reperfusion injury.

HDL THERAPY: MECHANISMS AND CLINICAL POTENTIAL

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The apolipoprotein A-I_{Milano} was a chance discovery in an individual with extremely low HDL cholesterolemia (7-10 mg/dl) from a family remarkably free from any vascular abnormalities. Extensive studies led to identification of the first mutant of human apolipoproteins. Apo A-I_{Milano} has a Cys for Arg replacement at position 173 of the aminoacid sequence in apo A-I. The dimeric form of apo A-I_{Milano} has a long permanence in blood and a high capacity of removing tissue cholesterol, in addition to attractive further properties (antiinflammatory, profibrinolytic, etc).

Availability of dimeric apo A-I_{Milano} opened the era of HDL therapy. This attempts to improve the vascular benefits exerted by other agents active on lipid metabolism, ie hypolipidemic drugs, also taking advantage of novel techniques of vascular evaluation, such as the intravascular ultrasound (IVUS) technology. HDL-liposomes with apo A-I_{Milano} have induced a rapid regression of a focal atheroma in an animal model (Chiesa et al, *Circ Res* 90: 974-980, 2002), as well as in patients with established coronary disease (Nissen et al, *JAMA* 290: 2292-2300, 2003). In this latter study, 5 weekly infusions (single doses as low as 1 g of protein) led to a remarkable reduction in the atheroma burden in a period of about 6 weeks, ie far better than reported in the REVERSAL Study with 18 months of continued statin administration (Nissen et al, *JAMA* 291: 1071-1080, 2004).

The mechanism of this very rapid and powerful effect is likely to be associated to the cholesterol removing capacity of the recombinant protein. A number of attempts to alternative developments in HDL therapy have been tested, from large unilamellar vesicles (LUV) of phospholipid liposomes, with the potential to directly remove free cholesterol from tissues, to analogues of the apo AI amphipathic helices. Non invasive methodologies are also being developed for evaluating atheromas, in order to provide earlier HDL therapy to patients with initial disease.

THE ROLE OF hsCRP IN DYSLIPIDEMIA

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Inflammation and hyperlipidemia both play crucial roles in all stages of atherogenesis including the conversion of stable to unstable plaques. Clinical application of the inflammation hypothesis has largely come from studies of high sensitivity C-reactive protein (hsCRP), an inflammatory biomarker that has consistently been shown to predict vascular risk at all levels of LDL cholesterol, at all levels of the Framingham Risk Score, and at all levels of the metabolic syndrome. Most recently, algorithms to incorporate hsCRP into global risk prediction models have been presented and demonstrate not only improved accuracy, but that 20 percent of apparently healthy individuals may need to be reclassified in terms of observed risk. hsCRP levels are also predictive of type 2 diabetes and adding an hsCRP value of > 3 mg/L to the formal definition of metabolic syndrome improves prediction not only of cardiovascular events, but also of diabetes. In secondary prevention, elevated hsCRP levels indicate high risk for recurrent disease independently of lipid levels, including not only LDL-C, and nonHDL-C, but also the apoB:apoA ratio. Further, statins lower hsCRP levels and both the PROVE-IT TIMI 22 trial and the REVERSAL study suggest that best clinical care following statin therapy occurs among patients who not only lower LDL-C, but also lower hsCRP levels. These data have lead to the hypothesis that the "dual targets" of LDL-C < 70 mg/dL and hsCRP < 2 mg/L may need to be implemented in high-risk secondary prevention. The role of statin therapy in primary prevention among individuals with low levels of LDL-C but elevated hsCRP levels is being actively investigated in the ongoing JUPITER trial. New data implicates multiple polymorphisms within the CRP gene as determinants of plasma CRP levels. Whether CRP plays a direct role in plaque rupture and thus represents a potential target for therapy remains investigative.

HDL AND REVERSAL OF MACROPHAGE FOAM CELL FORMATION

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Plasma high density lipoprotein (HDL) levels are inversely related to atherosclerosis risk. A major hypothesis is that HDL or its apolipoproteins promotes the efflux of cholesterol from cells in the arterial wall, cholesterol transport in plasma and excretion in the liver i.e. reverse cholesterol transport (RCT). The recent elucidation of key molecular players in the RCT pathway has provided strong support for this hypothesis. Many of the molecules mediating individual steps of RCT are controlled by the oxysterol-activated LXR transcription factors and are thus up-regulated in cholesterol loaded cells. This includes ABCA1, the defective molecule in Tangier Disease, that promotes cholesterol efflux to lipid-poor apoA-I. ABCA1 has a key role in the regulation of HDL levels and in the efflux of cholesterol from macrophage foam cells. However, ABCA1 interacts poorly with the major plasma HDL fractions. Recently, we showed that another transporter, ABCG1, promotes cholesterol efflux to HDL, but not to lipid-poor apoA-I. ABCG1 is an LXR and possibly a ppar-gamma target, is highly expressed in macrophages, and is responsible for the LXR-stimulated cholesterol efflux to HDL in macrophages. It is possible that the activity of ABCG1 is responsible for the anti-atherogenic properties of the HDL fraction. Recent work in ABCG1-/- mice shows neutral lipid accumulation in lung, spleen, liver and other tissues in animals fed high fat, high cholesterol diets and atherosclerosis studies are underway. The mechanism of action of ABCG1 is unclear, as it does not appear to directly bind HDL. In the future it may be possible to develop drugs that up-regulate relevant ABC transporters as well as HDL levels, such as LXR or ppar-gamma activators.

CV BIOMARKERS AND CAD PROGRESSION: THE LINK BETWEEN LDL-C, CRP, AND ATHEROSCLEROSIS

Steven E. Nissen, MD

Background: Recent trials have demonstrated better outcome with intensive than with moderate statin treatment. Intensive treatment produced greater reductions in both low-density lipoprotein cholesterol (LDL-C) and C-reactive protein (CRP), suggesting a relationship between these two biomarkers and disease progression. **Methods:** Intravascular ultrasonography was performed 502 patients with angiographically documented coronary disease. Patients were assigned to receive moderate treatment (40mg of pravastatin orally per day) or intensive treatment (80mg of atorvastatin orally per day). Ultrasonography was repeated after 18months to measure the progression of atherosclerosis. Lipoprotein and CRP levels were measured at baseline and followup. Results: for the group as a whole, the mean LDL-C level was reduced from 150.2mg per deciliter (3.88 mmol per liter) at baseline to 94.5 mg per deciliter (2.44 mmol per liter) at 18months (p<0.001), and the geometric mean CRP level decreased from 2.9 to 2.3 mg per liter (p<0.001). The correlation between the reduction in LDL-C levels and that in CRP levels was weak but significant in the group as a whole (r=0.13, p=0.005), but not in either treatment group alone. In univariate analyses, the percent change in the levels of LDL-C, CRP, apolipoprotein B-100, and non-high density lipoprotein cholesterol were related to the rate of progression of atherosclerosis. After adjustment for the reduction in these lipid levels, the decrease in CRP levels was independently and significantly correlated with the rate of progression. Patients with reductions in both LDL-C and CRP that were greater than the median had significantly slower rates of progression than patients with reductions in both biomarkers that were less than the median (p=0.001). **Conclusions:** intravascular ultrasonography provides a precise and continuous measure of the progression of atherosclerosis. The reduced rate of progression of atherosclerosis associated with intensive statin treatment is significantly related to greater reductions in the levels of both atherogenic lipoproteins and CRP.

1. Nissen SE, Tuzcu EM, Schoenhagen P. Statin therapy, LDL Cholesterol, C-Reactive Protein, and coronary artery disease. *N Engl J Med* 2005;352:29-38.

ANTI-INFLAMMATORY EFFECTS OF STATINS

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Statin therapy mediates its cardioprotective effects through modulation of endothelial function, inflammatory responses, plaque stability and thrombus formation, processes involved in atherosclerosis. Although reduction in low-density lipoprotein cholesterol (LDL-C) potentially plays a role in all of these effects, several lines of evidence also implicate non-lipid-mediated 'pleiotropic' effects. For example: 1) Statin therapy confers a lower risk for coronary artery disease (CAD) than placebo in patients with comparable serum cholesterol levels and confers a greater magnitude of clinical benefit than expected based on LDL-C levels alone. 2) Statins improve endothelial function in human subjects even before plasma LDL-C reduction is detected and more than ezetimibe for equal magnitude of LDL-C lowering. 3) In monkeys with atherosclerosis induced by dietary hypercholesterolemia, statins improve features associated with plaque stability even when cholesterol is clamped at a constant level by dietary manipulation. 4) Reduction in C-reactive protein (CRP), a marker of inflammation, with statins in humans with atherosclerosis is independent of the magnitude of LDL-C reduction. 5) Moreover, while non-statin lipid-lowering therapy does not necessarily reduce stroke risk, statins have shown a consistent reduction in stroke. Statins exert their pleiotropic effects on stroke, in part, by improving endothelial function and preventing platelet aggregation.

Accumulating evidence suggests that overactivity of the Rho and RAS signaling pathways plays a key role in atherosclerosis and many of the associated cellular dysfunctions that characterize this disease, i.e. vascular inflammation, smooth muscle cell proliferation, vasoconstriction and a procoagulant state. Statins inhibit the Rho and RAS pathways in experimental studies and exert their 'pleiotropic' vascular effects through this mechanism. Understanding of the biological effects of statins should lead to a more optimal utilization of this important class of therapeutic agents.

RATIONALE AND DESIGN FOR A LARGE-SCALE STUDY OF FIBRATE THERAPY IN DIABETES: THE FIELD TRIAL

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Randomized trials of fibrate therapy have so far examined only around 2,000 patients with diabetes, compared with evidence in over 18,000 such persons in trials of statin therapy. As fibrates are a logical treatment in diabetes, where HDL cholesterol levels are often low, and TG levels increased, the FIELD trial is designed to explore this issue.

FIELD is a double-blind randomized controlled trial to determine whether, among people with diabetes, long-term use of fenofibrate reduces major cardiovascular events. A total of 9795 patients with type 2 diabetes (50-75 years) have been randomized to either daily comiconised fenofibrate 200 mg or matching placebo for not less than 5 years on average. Eligible patients had a total cholesterol (TC) level between 115-250mg/dL (3.0-6.5 mmol/L) at entry, plus either a TC:HDLc ratio >4.0 or triglyceride >88mg/dL (1.0mmol/L).

Baseline characteristics of 9,795 patients randomised into FIELD

Characteristic (% or median)	Males	Females
Age >= 65 years	42%	38%
Current smoker	10%	8%
BMI >=30 kg/m ²	42%	60%
History of hypertension	52%	64%
Receiving insulin	14%	13%
TC (mg/dL)	192	204
HDLc (mg/dL)	38	46
TG (mg/dL)	150	159
HbA1c	6.9%	6.9%

Changes in lipids compare favourably with those seen in other fibrate trials, with a reduction of about 10% in TC and LDLc, 25% in TG and 6.5% increase in HDLc over 6 weeks of active run-in. FIELD is the largest primary prevention study in type 2 diabetes to date. Median follow-up is now 5 years, with over 500 major coronary events (fatal CHD plus non-fatal MI). Allocation to fenofibrate is rapidly associated with substantial beneficial changes in the lipid profile. It will clarify the indications for using a fibrate in type 2 diabetes. The study will report later this year.

OPTIMAL MANAGEMENT OF CVD RISK IN PATIENTS WITH TYPE 2 DIABETES AND THE METABOLIC SYNDROME

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Numerous major clinical trials conducted over the past decade have reported that pharmacotherapy aiming at the lowering of total cholesterol or LDL-cholesterol levels with statins reduces the risk of a first or recurrent CHD event. The ability of LDL-cholesterol concentration to adequately identify individuals at high risk for the development of CHD is limited as a considerable proportion of patients (as high as 50%) with established CHD may have cholesterol levels in the normal range. Thus, additional factors obviously modulate the risk of CHD associated with any given LDL-cholesterol concentration. For instance, it has been reported that the cluster of metabolic disturbances observed among individuals with abdominal obesity, the so-called metabolic syndrome, is associated with a substantially increased risk of CHD. In this regard, there is also increasing evidence that patients under statin therapy showing features of the metabolic syndrome with or without type 2 diabetes remain at increased CHD risk compared to patients without the metabolic syndrome. For instance, in the Heart Protection Study, the residual CHD event rate observed among patients with low HDL-cholesterol levels and under simvastatin therapy remained higher than the CHD event rate noted among patients with normal HDL-cholesterol levels who received a placebo. Therefore, these results support the notion that statin therapy does not normalize the risk of CHD associated with the metabolic syndrome with or without type 2 diabetes. These results provide a striking example that we need to go beyond targeting LDL-cholesterol lowering for the optimal management of CHD risk in abdominally obese individuals with features of the metabolic syndrome or type 2 diabetes. For these patients, in addition to the management of traditional risk factors, targeting abdominal obesity by reshaping nutritional and physical activity habits and managing other features of the metabolic syndrome such as the high triglyceride-low HDL-cholesterol dyslipidemia and inflammation may yield additional clinical benefits.

PPAR-ALPHA AGONISTS: MECHANISMS OF ACTION AND PARTICULAR BENEFITS IN PATIENTS WITH TYPE 2 DIABETES AND THE METABOLIC SYNDROME

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We now appreciate that patients with type 2 diabetes and/or the metabolic syndrome have heightened inflammation, a driver of all stages of atherosclerosis. For example, such individuals have high levels of plasminogen activator inhibitor -1 and C-reactive protein. In addition, although diabetic patients undoubtedly benefit from statin treatment, many such individuals have elevated triglycerides, and low HDL levels, aspects of diabetic dyslipidemia incompletely addressed by this class of agents. Certain polyunsaturated fatty acids can limit endothelial inflammation *in vitro*, and a Mediterranean diet can reduce coronary risk without substantially altering levels of LDL. These observations prompted us to evaluate potential anti-inflammatory effects of PPARs in atherosclerosis some years ago. Indeed, PPAR- α agonism can limit cytokine-induced activation of vascular endothelial cells, including expression of vascular cell adhesion molecule-1 and the procoagulant tissue factor. In addition PPAR-alpha agonism addresses the features of diabetic dyslipidemia beyond LDL. Thus, by mechanisms that attack both traditional lipid targets and more recently recognized inflammatory pathways, PPAR-alpha agonists such as the fibrates may mitigate atherosclerosis in diabetic patients, a proposition currently being addressed by large scale clinical trials.

ASSESSING THE EFFICACY AND SAFETY OF FIBRATE-STATIN COMBINATION THERAPIES IN THE MANAGEMENT OF HIGH-RISK MIXED DYSLIPIDEMIC PATIENTS

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A large body of randomized clinical trial data in the primary and secondary prevention of coronary heart disease (CHD) supports the monotherapy use of statins for reduction of CHD events and stroke. This benefit occurs with a very low incidence of adverse events, in a wide range of patients at moderate to high global risk, regardless of age, gender or baseline low density lipoprotein (LDL) cholesterol levels. Recent data suggests that more intensive reductions in LDL-C are more beneficial than less intensive therapy with statins in high risk subjects. Combination therapy with a statin and fibrate in individuals with a mixed dyslipidemia results in additive benefits on plasma lipid levels, and is an attractive therapeutic option. Reports of adverse events, particularly myositis, or more rarely, rhabdomyolysis have been known for many years, especially with gemfibrozil and statins. Recent pharmacokinetic data suggests that gemfibrozil, but not fenofibrate, interfere with statin glucuronidation, resulting in higher than expected statin plasma levels. Although the cause of statin myopathy is not known, high statin plasma levels are frequently associated with the myopathy risk. This presentation will evaluate the efficacy and safety issues of combination statin-fibrate treatment in high-risk mixed dyslipidemia patients.

SYNTHETIC HDL FOR CHD PREVENTION AND TREATMENT

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Several lines of evidence support the concept that increasing the plasma concentration of high density lipoproteins (HDL) may provide protection against coronary heart disease (CHD), and HDL have emerged as a new potential therapeutic target to complement LDL-lowering treatments in the attempt of further reducing CHD. Small, orally active molecules targeting factors involved in the regulation of HDL metabolism are under development as drugs to chronically raise plasma HDL levels. A distinct approach is based on the acute or sub-acute administration of synthetic HDL (sHDL). Differently from plasma HDL, which are heterogeneous in shape, size, and composition, sHDL are homogeneous particles made of two components: a phosphatidylcholine and a single apolipoprotein, either apoA-I, an apoA-I variant (like apoA-IMilano), or an apoA-I mimetic peptide. Such HDL were shown to mimic most of the functions of plasma HDL. The efficacy of sHDL in inducing the regression of atherosclerosis has been tested in animals and patients with established atherosclerotic plaques given single or repeated injections of sHDL containing the A-IMilano dimer. In rabbits treated with high doses of such sHDL, a single 90 min infusion reduced the plaque area up to 30%; in coronary patients, 5 weekly infusions of sHDL at relatively low doses caused a 4.2% reduction of total atheroma volume. Additional clinical trials are required to establish whether short-term treatments with sHDL will prevent acute coronary events. The multiple beneficial effects of sHDL and the evidence from *in vivo* studies in animals support the concept that the therapeutic potential of sHDL may extend beyond the regression of atherosclerosis and the prevention of its consequences. sHDL limit cardiac dysfunction and cardiomyocyte damage after ischemia/reperfusion (I/R) injury *ex vivo* and *in vivo*. Thus, sHDL may become a clinically useful form of treatment in various conditions characterized by I/R injury, such as acute coronary syndromes, revascularization procedures, or cardiac surgery.

CHOLESTEROL ESTER TRANSFER PROTEIN INHIBITION, LIPID METABOLISM AND HEART DISEASE PREVENTION

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Cholesterol ester transfer protein (CETP) facilitates the exchange of cholesteryl ester in HDL for triglyceride. Animals resistant to diet induced atherosclerosis such as mice and rats lack CETP, and have HDL as their major lipoprotein, in contrast to hamsters, rabbits, monkeys, and humans. Treating rabbits on atherogenic diets with a CETP inhibitor decreases atherosclerosis compared to control animals. The benefits of CETP inhibition in humans has been debated because of the observation that one kindred with combined CETP and hepatic lipase deficiency had premature CHD, and that heterozygotes with CETP deficiency in the Honolulu Heart Study had increased CHD risk. However other studies in Japan have not documented premature CHD in homozygous CETP deficiency, and a reanalysis of the Honolulu Heart Study did not confirm the original findings, and in fact indicated that the converse was true. Moreover probucol which raises CETP activity and lowers HDL did not have benefit in the treatment of femoral atherosclerosis in humans in a randomized controlled trial. Our own studies in Framingham and VA-HIT indicate that about 20% of the population are homozygous for the B2 allele at the Taq1B site within the CETP gene which is in strong linkage disequilibrium with a promoter variant which decreases CETP activity, increases HDL C, and is associated with a significant reduction in CHD risk. Moreover our studies in healthy Ashkenazi Jews of mean age 98 years and their offspring compared to controls documented a 4 fold increased prevalence of homozygosity for I405V allele at the CETP locus. We have tested the CETP inhibitor tocetrapib and shown that it is extremely effective in raising HDL cholesterol by more than 50% in patients with HDL deficiency, and that it has beneficial effects on HDL particle distribution, and lipoprotein metabolism. Clinical trials are underway, which we predict will be very positive.

INFLAMMATION IMPAIRS THE ABILITY OF MOUSE SERUM TO REMOVE CELLULAR LIPIDS BY THE ABCA1 PATHWAY

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HDL is an atheroprotective lipoprotein that removes cholesterol from peripheral cells via the ABCA1 and ABCG1 transporters. Inflammation decreases the atheroprotective effects of HDL by reducing its apoA-I and PON-1 levels and increasing its SAA composition. Here we studied the effects of inflammation on the ability of serum HDL particles to remove cellular lipids. Inflammation was induced by LPS injection (100mg/mouse, *i.p.*) into C57BL/6 mice. After 24 hours, serum (for lipid efflux) and plasma (for lipid measurements) were prepared immediately. Plasma levels of total cholesterol, triglycerides and SAA were significantly increased by LPS injection, but HDL cholesterol did not change. To test the effects of inflammation on the activity of serum particles that remove cellular lipids by the ABCA1 or ABCG1 pathways, we measured radiolabeled cellular cholesterol and phospholipid efflux into serum using baby hamster kidney cells transfected with inducible cDNAs for each of these transporters. Cholesterol efflux into 5 % serum increased only 10-20 % when either ABCA1 or ABCG1 were induced, indicating that most of the cholesterol efflux promoted by mouse serum occurs by processes independent of these transporters. In contrast, inducing ABCA1 increased phospholipid efflux into serum 3-fold, indicating that efflux of cellular phospholipids into mouse serum depends mostly on ABCA1. Treating animals with LPS significantly reduced the ABCA1-dependent cholesterol and phospholipid efflux activity of serum but had no effect on ABCG1-dependent cholesterol efflux. Thus, LPS-induced inflammation selectively reduced or impaired particles in serum that remove cellular lipids by the ABCA1 pathway. The findings raise the possibility that reduced ABCA1-dependent cholesterol efflux from arterial macrophages contributes to the enhanced atherogenesis associated with inflammatory disorders.

HIGH-DENSITY LIPOPROTEINS INDUCE TRANSFORMING GROWTH FACTOR BETA 2 EXPRESSION IN ENDOTHELIAL CELLS

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Background. HDL are endowed with cardiovascular protective activities. In addition to their role in reverse cholesterol transport, HDL influence different functions of endothelial cells. In the present study we investigated in endothelial cells the genes involved in inflammation modulated by HDL.

Methods and Results. Using cDNA array analysis transforming growth factor (TGF) β 2 appeared as a gene responsive to HDL treatment in endothelial cells. Quantitative real time PCR experiment confirmed that HDL subfraction 3 selectively induces TGF- β 2 mRNA expression and protein release while TGF- β 1 and TGF- β 3 were not affected. This effect was mainly PI3K/Akt dependent. Lysosphingolipids present in HDL such as sphingosine 1 phosphate and sphingosylphosphorylcholine mimicked the effects of the whole HDL. These results were confirmed in vivo in transgenic mice overexpressing human apoA-I. Compared with apoA-I knock-out mice, phospho-Akt, phospho-ERK1/2 and TGF- β 2 expressions were increased in the aorta of transgenic mice overexpressing human apoA-I. In addition, the expression of phospho SMAD2/3, the transcription factor activated by TGF- β , is increased in transgenic mice compared to knock-out mice.

Conclusion. Since TGF- β possess anti-inflammatory properties and stabilizes the plaque, the results of the present work suggest a novel target for the anti-atherosclerotic effect of HDL.

SELECTIVE MODIFICATION OF HDL APOLIPOPROTEIN COMPOSITION BY CONJUGATED LINOLEIC ACID ISOMERS IN APOLIPOPROTEIN E KNOCKOUT MICE

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An inverse relationship is observed between triglycerides and HDL cholesterol. To explore potential mechanisms involved in such association, mice deficient apo E, thereby lacking CETP activity, were made hypertriglyceridemic by feeding them conjugated linoleic acid (CLA) isomers in a Western-type diet and apolipoprotein components of HDL as well as apolipoprotein C-III levels were assayed. Despite the pronounced hypertriglyceridemia observed in these animals following the administration of the *trans*-10, *cis*12- CLA isomer, HDL cholesterol was significantly increased. No changes in plasma concentration and distribution of apolipoprotein A-IV were observed. However, apolipoprotein A-I concentration in the latter group significantly decreased in comparison with *cis*-9, *trans*-11- CLA isomer group. The decrease in this plasma concentration was associated with decreased hepatic expression and a shift of the apolipoprotein to lipid-poor particles. Plasma apolipoprotein A-II concentration increased and was preferentially distributed into HDL in animals consuming the *trans*-10, *cis*12- CLA isomer, thereby justifying the increase in HDL cholesterol. An inverse association ($r = -0.73$, $p < 0.001$) between the plasma concentration of both apolipoproteins A-I and A-II was observed. A significant and positive association ($r = 0.88$, $p < 0.001$) was also found between apolipoprotein A-II and C-III. These results indicate that in absence of CETP, the presence of apolipoprotein A-I and A-II in HDL together with the levels of apo C-III are important determinants of the relationship between triglycerides and HDL cholesterol.

PDZK1 C-TERMINAL REGION, NOT DIRECTLY INVOLVED IN SR-BI BINDING, IS REQUIRED FOR UP-REGULATION OF SR-BI EXPRESSION.

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Scavenger receptor class B type I (SR-BI) is a high-density lipoprotein (HDL) receptor that mediates the selective uptake of HDL cholesterol and cholesterol secretion into bile in the liver. We previously identified an SR-BI-associated protein from rat liver membrane extracts, now termed PDZK1. PDZK1 contains four PDZ domains, the first of which in the N-terminal region is responsible for the association with SR-BI. PDZK1 controls hepatic SR-BI expression in a post-transcriptional fashion both in cell culture and *in vivo*. In this study, we demonstrated that the C-terminal region of PDZK1 is crucial for up-regulating SR-BI protein expression. CHO-K1 cells expressing various deletion mutants of PDZK1 were established and transiently transfected with SR-BI. SR-BI protein expression was increased 4- to 5-fold in the cells co-expressing SR-BI and full length PDZK1. It was also found that any PDZK1 deletion mutant lacking the C-terminal domain was unable to up-regulate the SR-BI protein. Even the mutant lacking only 66 amino acids at the C-terminal was less effective compared with the full-length PDZK1. These results suggest that PDZK1 is capable of up-regulating the SR-BI protein and that a region within the C-terminal 66 amino acids, not directly involved in SR-BI binding, is required for this activity.

THERAPEUTIC INTERVENTIONS INTENDED TO PREVENT THE PROGRESSION OF ACUTE CORONARY SYNDROME USING RECONSTITUTED HIGH-DENSITY LIPOPROTEIN CHOLESTEROL

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Although drug-eluting stents have been shown to dramatically reduce neointimal growth, which can lead to a marked reduction in restenosis rates, it remains unclear how to prevent initial coronary atherosclerosis. There is a strong inverse relation between the risk of acute coronary syndrome (ACS) and high-density lipoproteins (HDLs). Recently, a reconstituted HDL (rHDL) called ApoA-I Milano/phospholipid complex was shown to produce a rapid regression of coronary atherosclerosis in patients with ACS. HDL takes up cellular cholesterol from the periphery and also has pleiotropic effects, in that it promotes anti-inflammation and anti-coagulation and protects against coronary artery disease. We reported that HDL and sphingosine-1-phosphate (S1P) induced potent signals through a Ras/extracellular-signal-regulated kinase (ERK) 1/2 pathway mediated by pertussis toxin-sensitive G-protein coupled receptor to promote angiogenesis in human coronary endothelial cells. In addition, we found that treatment with the rHDL POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine)/ApoA-I, which we reconstituted, prevents reperfusion-induced ventricular fibrillation and tachycardia through an increase in nitric oxide production and pathological cardiac remodeling through ERK activation in a rat model of myocardial infarction. We also examined the role of a newly developed rHDL, POPC/S1P/ApoA-I. It affected cell survival, including cell proliferation and coronary endothelial tube formation, through ERK activation independent of a Ras pathway, in addition to cholesterol efflux in an *in vitro* study. These findings represent an exciting new area in coronary atherosclerosis intervention. HDL-based therapy combined with the reduction of low-density lipoproteins may be able to dramatically reduce the incidence of ACS as well as aid in the recovery from ACS.

NUCLEAR RECEPTORS MODULATING THE METABOLIC SYNDROME: FOCUS ON PPAR α AND FXR.

B. Staels

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Cardiovascular disease is significantly increased in patients with the metabolic syndrome and type 2 diabetes. A clustering of risk factors, including dyslipidemia, insulin resistance, hypertension, inflammation and coagulation disorders result in an increased risk for cardiovascular events in these patients. The Farnesoid X Receptor (FXR) and peroxisome proliferator-activated receptor (PPAR) α are members of the nuclear receptor superfamily. Whereas PPAR α is activated by fatty acids, FXR has recently been identified as a bile acid-activated nuclear receptor. FXR not only controls bile acid synthesis, conjugation and transport, but also lipid and glucose metabolism. Activation of PPAR α represents one important pathway that influences vascular function both directly and indirectly. PPAR α activation induces beneficial effects not only on lipid metabolism, but also influences glucose homeostasis, endothelial function and vessel wall inflammation. PPAR α agonists in clinical use, such as fibrates, may alter the process of atherosclerosis, especially in subjects with the metabolic syndrome and type 2 diabetes. This presentation will highlight the molecular mechanisms of FXR and PPAR α action in the prevention and treatment of the metabolic syndrome and atherosclerosis.

A CELL BASED ASSAY PLATFORM FOR THE HIGH THROUGHPUT SCREENING OF LIGAND GATED TRANSCRIPTION FACTORS AGONISTS

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Nuclear hormone receptors (NHRs) form a superfamily of ligand-activated transcription factors, which currently represent a major targets or drug discovery in cardiovascular diseases. Among them: PPARs play key role in lipid and lipoprotein metabolism, metabolic syndrome, and glucose homeostasis, Liver X receptors (LXRs) are master transcription factors regulating cholesterol and fatty acid metabolism, while Estrogen receptors are involved in atherosclerotic processes.

Taking advantage of the modular structure of the NHRs, we have developed a proprietary platform for high throughput screening of molecules able to bind and activate NHRs. We have generated chimeric receptors comprising the LAC9 transcription factor DBD fused to the LBDs of PPAR α , PPAR δ , PPAR γ , ER α , ER β , LXR α and LXR β . In parallel we have cloned a vector containing the LAC9 response elements operatively linked to the reporter gene luciferase. We have transfected in a permanent way the LAC9-luciferase vector into CHO-K1 cell line to generate a general reporter cell line and we have subsequently transfected in a stable way the LAC9(DBD)-NHR(LBD) vectors. The cell lines have been validated by testing the specific agonists and optimized for the 96, 384 and 1536 MTP formats; showing strong luciferase induction after 6 hours incubation in the presence of the ligands.

This cell based platform represents a robust, reliable, fast, highly sensitive and high throughput system for the screening and identification of specific NHR agonists.

MECHANISM OF ACTION OF PPAR γ AGONISTS IN HUMANS

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The insulin sensitizing thiazolidinediones (TZDs), which are selective ligands of the nuclear transcription factor peroxisome proliferator-activated receptor (PPAR) γ , are the first drugs of this class that address the basic problem of insulin resistance in patients with type 2 diabetes. Furthermore, this class of agents may possibly have a role in treating non-diabetic insulin resistant conditions. Therapeutic doses of PPAR γ agonists have been shown to change transcription of at least 30 genes in humans. In contrast to studies in animals, data regarding the mechanism of action of TZDs in humans are sparse. Both of the currently available TZDs, pioglitazone and rosiglitazone, increase hepatic insulin sensitivity, and have in 8 human studies been shown to reduce liver fat content by 50%. Since insulin lowers blood glucose by inhibiting hepatic glucose production rather than by stimulating glucose uptake in type 2 diabetes (the mass action effect of glucose compensates for insulin resistance and maintains glucose flux normal), the hepatic effects of TZDs are of obvious importance. It is less clear how hepatic insulin sensitivity improves by TZDs since normally PPAR γ receptors are abundantly expressed in adipose tissue rather than the liver. TZDs in humans dramatically (2-4 fold) increase adiponectin concentrations via an effect on adiponectin gene expression in adipose tissue. Changes in adiponectin correlate closely with those in liver fat. Pioglitazone and rosiglitazone have different effects on lipoprotein metabolism but their mechanisms of action on lipoprotein metabolism have not been compared. Whether the net effect of multiple actions of TZDs on glucose, and lipid metabolism, adipokines and markers of inflammation have favourable effects on CV end points is still unclear. Results of the PROACTIVE study, which addresses effects of pioglitazone on CV events and will be presented in Athens 2005 are awaited with interest.

LXR β REGULATION IN LXR α DEFICIENT MICE: IMPLICATIONS FOR THERAPEUTIC TARGETING.

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The nuclear receptors LXR α and LXR β are differentially expressed ligand-activated transcription factors that induce genes controlling cholesterol homeostasis and lipogenesis. Synthetic ligands for both receptor subtypes activate ABCA1-mediated cholesterol metabolism, increase reverse cholesterol transport and provide atheroprotection in mice. However, these ligands may also increase hepatic triglyceride (TG) synthesis via SREBP-1c-dependent stimulation, a process reportedly governed by LXR α . We studied pan LXR α/β agonists in LXR α $-/-$ knockout mice to assess the contribution of LXR β to regulation of selected target genes. *In vitro* studies with macrophages from LXR α $-/-$ and β $-/-$ mice reveal an equivalent role for LXR α and LXR β in the regulation of ABCA1 and SREBP gene expression.

The *in vivo* role of LXR β in liver was further evaluated by treating LXR α $-/-$ mice with a pan LXR α/β agonist. HDL-cholesterol increased without significant changes in plasma TG or VLDL. Analysis of hepatic gene expression revealed less activation of ABCA1 and SREBP-1c genes in the liver of α null animals than in treated-WT controls. In addition, hepatic Cyp7a1 and several genes involved in fatty acid/ TG biosynthesis were not induced. In peripheral tissues from these mice, duodenum, kidney, and spleen, LXR β activation impacts ABCA1 and SREBP-1c gene expression in a parallel manner. However, putative elevation of SREBP-1c activity in these tissues did not cause hypertriglyceridemia. In summary, selective LXR β activation is expected to stimulate ABCA1 gene expression in macrophages, contribute to favorable HDL increases, and circumvent hepatic LXR α -dominated lipogenesis.

THERAPEUTIC SILENCING OF APOB BY SYSTEMIC DELIVERY OF SHORT INTERFERING RNAS

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Novel therapies based on short interfering RNA (siRNA) duplexes have tremendous potential to treat diseases by silencing the expression of otherwise non-druggable proteins. A single intravenous administration of cholesterol conjugated siRNA duplexes (chol-siRNA) targeting Apolipoprotein B (ApoB) results in significant inhibition of target gene expression in the liver and intestine of mice. We also show that silencing is dose-dependent and that silencing in liver lasts longer than one week. Extending these findings, we modified potential endo- and exonuclease cleavage sites and thereby improved in vitro serum half live of siRNA duplexes up to 20 hours (or 50-fold), and enhanced in vivo efficacy which lead to a siRNA compound that silenced apoB mRNA expression in the gut by 80%. In addition we characterized the ability of chol-ApoB-siRNA to reduce cholesterol levels in mice fed a high-fat ("Western-type") diet. In this model, we show that silencing of ApoB by repeated administration of chol-siRNA reduces total cholesterol to nearly normal levels. The continuous reduction of apoB mRNA in liver mimicked a lipid accumulation phenotype that is seen in mice with familial hypobetalipoproteinemia. These results represent a significant advance in the development of systemic siRNA therapeutics.

APOLIPOPROTEIN A5 ACCELERATES PLASMA HYDROLYSIS OF TRIGLYCERIDE-RICH LIPOPROTEINS BY INTERACTION WITH PROTEOGLYCAN BOUND LIPOPROTEIN LIPASE

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Apolipoprotein A5 (apoA5) is associated with changes in triglyceride levels and with familial combined hyperlipidemia. To elucidate the mechanism by which apoA5 influences plasma triglycerides, metabolic studies and in vitro assays resembling physiological conditions were performed. In apoA5 transgenic mice (apoA5^{tr}), catabolism of chylomicrons and VLDL was accelerated due to a faster plasma hydrolysis of triglycerides by lipoprotein lipase (LPL). Hepatic VLDL and intestinal chylomicron production were not affected. The functional relation between apoA5 and LPL was further investigated by crossbreeding a human LPL transgene with the apoA5 knockout, and apoA5^{tr} mice to an LPL deficient background. Increased LPL activity completely normalized hypertriglyceridemia of apoA5 deficient mice, however, overexpression of human apoA5 only slightly modulated triglyceride levels when LPL was reduced.

To reflect the physiological situation in which LPL is bound to cell surface proteoglycans, LPL mediated hydrolysis of triglyceride rich lipoproteins was examined in presence and absence of proteoglycans. Without proteoglycans, apoA5 derived either from triglyceride-rich lipoproteins, apoA5^{tr} HDL, or a recombinant source did not alter LPL hydrolysis rates. In the presence of proteoglycans, however, apoA5 led to a significant and dose-dependent increase in LPL mediated hydrolysis of VLDL triglycerides. These results were confirmed in cell culture using a proteoglycan-deficient cell line.

In summary, it is proposed that apoA5 reduces triglyceride levels by guiding VLDL and chylomicrons to proteoglycans bound LPL for lipolysis.

INFLUENCE OF THE COMMON POLYMORPHISMS ON THE TREATMENT OF METABOLIC SYNDROME

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Genetic variants of the lipoprotein lipase, apolipoprotein E and CIII, PPARalpha, beta3 adrenergic receptors genes have been suggested to be associated with metabolic syndrome.

In our study we evaluated the influence of the common lipoprotein lipase gene (N291S), beta3 adrenergic receptors gene (Trp64Arg), apolipoprotein CIII (S1S2), PPARalpha gene (L162V) polymorphisms and the genotypes of apolipoprotein E (ApoE) on lipid and carbohydrate metabolism in the 144 patients: 74 obese patients with metabolic syndrome and 70 healthy participants.

The incidence of the polymorphism of the N291S, Trp64Arg, S1S2 and e2, e3, e4 alleles in our patients did not differ with other European Population, but frequencies for L162V in our group was significantly higher (p=0,016).

An associations between anthropometrical parameters, serum lipoproteins; insulinemia, glycemia fasting and after 2 hours OGTT between carriers and no carriers of the polymorphic sites was observed. The patients who were carriers of the L162V with Trp64Arg, S1S2 and e4 allele carriers were responded poorly to diet with metformin treatment in weight loss and atherogenic lipoprotein levels. So they needed lipid lowering drugs.

The Trp64Arg carriers showed good response to metformin and diet treatment and did not need lipid lowering drugs therapy.

We conclude that common genes polymorphisms of the PPARalpha, beta3 adrenoreceptors, Apo C III and apolipoprotein E investigation are useful additional guide to treatment of the patients with metabolic syndrome.

AN OVERVIEW OF RECENT ARTICLES ON TRIGLYCERIDES AND METABOLIC SYNDROME

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The recent literature on the metabolic syndrome focuses on diagnosis, level of associated cardiovascular risk, ethnic differences, metabolism and therapy. Reviews have appeared on its definition, pathophysiology and mechanisms as well as on the evaluation of pathological and therapeutic outcomes. The syndrome definition was updated by the International Diabetic Federation. A high prevalence of calcified atherosclerotic plaque in the coronary arteries and abdominal aorta of white and African-American men and women measured by tomographic scanning was observed in the metabolic syndrome. Low household income and use of psychotropic drugs appear to increase the risk of this syndrome in women. Postprandial lipemia and hyperuricemia were found to be a function of apoE genotype in the metabolic syndrome. A large interest has been given to the inflammatory component of the syndrome and changes in adipose tissue leptin and adiponectin. An evolutionary link between hypertriglyceridemia and inflammation has been proposed and hepatic inflammation secondary to liver steatosis is now considered a potential contributor to the low-grade inflammation associated with the syndrome. Low adiponectin levels have been reported in the metabolic syndrome and several of its components in an urban south Indian population. The importance of visceral fat accumulation and the potential role of visfatin is also attracting much attention. In spite of a smaller reduction in weight and body fat on a weight reduction regimen, subjects with visceral obesity have greater improvements in parameters of the metabolic syndrome (fasting glucose, triglycerides and HOMA) than subjects with subcutaneous fat accumulation. Visfatin a visceral fat adipokine which mimics the effect of insulin was found to be negatively regulated by IL-6. From the therapeutic standpoint, diet, PPAR gamma agonists, metformin and lifestyle intervention, statins and CB1 receptor antagonists (RIO-Europe) are given significant positive coverage.

ADIPOCYTE LIPOLYSIS AND THE METABOLIC SYNDROME

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In obese people, triglycerides overaccumulated in adipose tissue spill over into the circulation as fatty acids, which stimulate VLDL production in the liver in turn. Thus, adipocyte lipolysis and its products may play a critical role in the development of some of the manifestations of the metabolic syndrome. Genetic ablation of hormone-sensitive lipase (HSL) in mice has unraveled new roles of this classical enzyme in adipocyte differentiation, food intake, and reproduction. These mice retain a substantial lipolytic activity in adipose tissue, suggesting the presence of a lipase(s) that is distinct from HSL. We have recently identified a novel triglyceride lipase whose expression is robustly induced in the adipose tissue during fasting, a condition known to be associated with increased adipocyte lipolysis. This presentation will highlight the recent advances in our understanding of adipocyte lipolysis and its clinical implication to the metabolic syndrome.

RELATIONSHIP BETWEEN TRIGLYCERIDE-RICH LIPOPROTEINS AND HDL

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An inverse relationship exists between levels of HDL-cholesterol (HDL-C) and triglyceride (TG). Whether intestinal production of TG and/or liver production of TG is responsible for this relationship is not known. Our purpose was to determine the relationship between metabolism of apolipoprotein (apo) A-I in HDL and apoB in TG including both TG-rich protein (apoB-48) in chylomicrons produced in the intestine and TG-rich protein (apoB-100) in very low density lipoprotein (VLDL) produced by the liver in moderately hypercholesterolemic humans. Methods: The kinetics of apoA-I within HDL, apoB-48 and apoB-100 within triglyceride-rich lipoproteins, and apoB-100 within intermediate-density lipoprotein and low density-lipoprotein (LDL) were examined with a primed constant infusion of [5,5,5-²H₃] leucine in the fed state (hourly feeding) in 23 subjects after consumption of a 36% total fat diet. Lipoproteins were isolated by ultracentrifugation and apolipoproteins by SDS-PAGE gels. Isotope enrichment was assessed by gas chromatograph/mass spectrometry. Kinetic parameters were calculated by multicompartamental modeling of the data with SAAM II. Results: ApoA-I production rate (PR) was correlated with LDL apoB-100 pool size (PS) ($r=0.49$; $P=0.017$) and LDL-C ($r=0.61$; $P=0.002$) whereas apoA-I fractional catabolic rate (FCR) was inversely correlated with apoB-48 FCR ($r=-0.40$; $P<0.05$) but not with VLDL apoB-100 FCR. In conclusion, two links exist between apoA-I and apoB kinetics: 1) when LDL apoB-100 PS is high, there is increased apoA-I PR; and 2) delayed chylomicron remnant clearance (represented by apoB-48 FCR) is associated with enhanced apoA-I FCR, a finding indicating that alterations in intestinal lipoproteins containing triglyceride-rich apoB-48 may be more important in determining HDL-C levels than changes in apoB-100 triglyceride-rich lipoproteins produced by the liver.

HIGH DENSITY LIPOPROTEINS IN METABOLIC SYNDROME: THE RELEVANCE OF TRIGLYCERIDE METABOLISM

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Anti-atherogenic actions of HDL include reverse cholesterol transport, antioxidant and anti-inflammatory activities. Small, dense HDL3 particles possess potent capacity to protect LDL against oxidative stress. It remains indeterminate however as to whether antioxidative activity of small, dense HDL is attenuated in atherogenic dyslipidemias.

Our data reveal that antioxidative activity of small, dense HDL3 is significantly impaired in dyslipidemic subjects presenting with metabolic syndrome (MetS, up to -23%) or Type 2 diabetes (up to -47%) as compared to normolipidemic controls. Systemic oxidative stress assessed as plasma 8-isoprostanes is significantly elevated in dyslipidemic subjects (3.7-fold in MetS, 2.9-fold in Type 2 diabetes) and is negatively correlated with antioxidative activity of small, dense HDL3. HDL particles from dyslipidemic subjects are triglyceride (TG)-enriched and cholesteryl ester (CE)-depleted; the deficient antioxidative activity of small, dense HDL3 is correlated with HDL core enrichment in TG and depletion of CE. When small, dense HDL3 are enriched in TG by *in vitro* incubation with TG-rich lipoproteins in the presence of CETP, antioxidative activity of HDL3 is completely abrogated. By contrast, selective inhibition of HDL enzymes possessing antioxidative properties (paraoxonase, PAF-AH) does not significantly influence antioxidative activity of HDL3 particles.

We conclude that in atherogenic dyslipidemias of MetS and Type 2 diabetes, replacement of CE by TG in the HDL particle core induces dysfunction in HDL antioxidative activity. We propose that CETP-mediated TG enrichment of HDL decreases the capacity of apoA-I to remove oxidised lipids from LDL, resulting in reduced antioxidative protection of LDL. Such TG-mediated attenuation of HDL anti-atherogenic activity may represent a key mechanism underlying the association of plasma TG levels with accelerated atherosclerosis.

TRIGLYCERIDE-RICH LIPOPROTEINS AND ENDOTHELIAL FUNCTION

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Triglyceride-rich lipoproteins (TGRLs) are a cardiovascular risk factor and induce endothelial dysfunction. We have investigated the biological basis of the effects of TGRLs from type IV hyperlipidemic and normolipidemic subjects on endothelial activation/ function focusing on their effects on intracellular pathways and gene expression.

TGRLs were isolated from hypertriglyceridemic (H-TGRL) and normotriglyceridemic (N-TGRL) subjects. RNA from human endothelial cells incubated with N-TGRL or H-TGRL was prepared for cDNA microarray analyses. Western blotting was used to study intracellular signaling pathways. Regulated genes were further studied with real-time PCR and immunofluorescence. Both N-TGRL and H-TGRL activated ERK1/2 and p38 MAPK. However, there were differences in the pattern of upregulated target genes between the two lipoprotein preparations: VCAM-1, PECAM-1 and PAI-1 were upregulated by both N-TGRL and H-TGRL, while ELAM-1, P-selectin, MCP-1, IL-6, TLR-4, CD40 and ADAMTS1 were selectively upregulated by H-TGRL. Chromatin immunoprecipitation analysis demonstrated the involvement of transcription factors NF- κ B and CREB in the activation of these genes.

Conclusion. These results support the involvement of hypertriglyceridemic TGRLs in endothelial dysfunction via induction of a pro-inflammatory and pro-thrombotic condition. These data set the stage for further addressing the role of TG rich lipoproteins in atherosclerosis.

TREATING LOW HDL-C AND THE CONSEQUENCES FOR LIPOPROTEIN FUNCTIONALITY AND CHD RISK

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The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial tested statin treatment in 70-82 year olds. Pravastatin lowered the risk of the CHD by 19% (P=0.006). Baseline LDL cholesterol was not associated with CHD risk. HDL cholesterol (HDLc) was however strongly, inversely related to CHD events (P=0.0002).

In contrast to other studies, statin efficacy exhibited an interaction with HDL (P = 0.007). Subjects in the top three quintiles showed no effect of treatment (RR=1.06, P=0.53) whereas those with HDLc<1.15mmol/l (45mg/dl) exhibited a 33% risk reduction (P<0.0001). Further, treatment abolished the association of HDLc with risk of a coronary event (HDLc vs CHD event in treated subjects P=0.25).

HDLc was weakly inversely related to C- reactive protein (r=-0.09, P<0.0001) and inclusion of CRP in multivariate models attenuated the relationship of HDLc to CHD risk (P=0.32) indicating that, in part, the deleterious effects of low HDL was linked to higher levels of inflammation.

Thus, in the elderly, HDL appears to be the main lipoprotein predictor of CHD risk, and identifies those who benefit most from statin therapy. An HDL-inflammation association may be key to understanding this outcome.

HDL ELEVATION THROUGH CETP INHIBITION: ANTI ATHEROGENIC?

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Two pharmacological small molecule inhibitors of CETP, JTT-705 and Torcetrapib have recently been shown to effectively raise HDL-c in humans without serious side effects when either used as monotherapy or combined with statins that lower LDL-c. Importantly, prospective data from the Epic-Norfolk study furthermore indicate that elevated CETP concentration in conjunction with elevated triglyceride levels are associated with increased odds for cardiovascular events. Data from the Diabetic Atherosclerosis Intervention Study (DAIS) also show that elevated CETP concentration is associated with increased progression of coronary atherosclerosis in patients with type 2 diabetes who use fenofibrate.

Long-term studies will have to show whether CETP inhibition decreases the risk of atherosclerotic disease in dyslipidemic patients. Increased CETP activity might be detrimental in particular under hypertriglyceridemic conditions, which is of importance when considering that a large proportion of patients at increased risk from CAD exhibit elevated triglyceride levels. Studies into the effects of CETP inhibition in hypertriglyceridemic patients therefore seem warranted. Awaiting the first data on the effect of CETP inhibition on surrogate endpoints for atherosclerosis, the complexity of HDL metabolism will necessitate a wide variety of studies on many aspects of this intriguing lipoprotein.

The effect of JTT 705 in familial hypoalphalipoproteinemia (FHA) will be discussed and will include NMR data, oxLDL antibody levels, small dense LDL concentration and ultimately the effect on the overall cardiovascular risk profile.

IS THE APPROPRIATE TARGET HDL OR TRIGLYCERIDES TO REDUCE EVENTS AND HOW DO WE GET THERE IN PATIENTS

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The blood plasma concentrations of both HDL and triglycerides (TG) are strong and continuous risk factors in community studies. An unfortunate debate has been kept alive due to the use of multivariate analyses that find TG to be a much weaker risk factor when associations with lower HDL cholesterol are taken into account. Biostatisticians remind us that one should not apply this statistical technique to observations that are linked through physiologic mechanisms as are VLDL and HDL metabolism. This type of analysis has led to the conclusion that HDL is an appropriate focus of treatment whereas triglycerides are a less worthy target. Furthermore, the weaker association between fasting plasma TG and clinical vascular events is in part due to the day to day variability of this measure. HDL values are more stable. Very few studies have made multiple observations in individuals to provide more accurate estimates of the "average" TG concentrations. Variability markedly weakens the power of statistical tools designed to establish correlations between continuous measures.

Subgroup analysis of the hypertriglyceridemic patients in clinical trials has consistently shown impressive benefit when fibrates have been the intervention. This benefit is accentuated when low HDL-C accompanied the elevated triglyceride. Unfortunately, there are no drugs that selectively raise HDL without significant reductions in TG and/or LDL. The data correlating vascular events with TG reduction or HDL-C elevation has been inconsistent. This has made the selection of a rationale target very difficult. The non-HDL cholesterol value in patients with higher TG is a rational, simple and low cost measure which allows one to set reasonable targets that are achievable with current treatment modalities. The scientific base for strengthening our clinical approach to this problem will be greatly advanced by clinical trials focusing on those with acceptable LDL but above average TG and below average HDL-C.

A NOVEL CELLULAR PHENOTYPE OF MACROPHAGES WITH DEFICIENCY OF ATP-BINDING CASSETTE TRANSPORTER-A1 (ABCA1)

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Background: It is well known that macrophages with ABCA1 deficiency present a defective lipid efflux and a subsequent accumulation of intracellular lipid droplets, called foam cell formation. However, phenotypes of these cells are still largely unknown.

Objective: We investigated whether ABCA1 deficiency influences the cholesterol-rich microdomains of outer plasma membrane with two newly developed probes both selectively binding to these domains; a protease-nicked and biotinylated derivative of perfringolysin O and a fluorescein ester of polyethylene glycol-derivatized cholesterol.

Results: Western blot analyses and the confocal laser scanning microscopy revealed that these two probes recognized a greater volume of cholesterol-rich microdomains in ABCA1-deficient macrophages and fibroblasts obtained from patients with Tangier disease and ABCA1 knockout mice. The phenotype was corrected by the gene introduction of ABCA1.

Conclusion: The ABCA1-deficient cells exhibited a novel phenotype, the alteration of cholesterol-rich microdomains. These results suggest that ABCA1 may regulate the formation and function of the important structure on the plasma membrane.

DOES GENETIC VARIATION IN *ABC TRANSPORTER A1* PREDICT ISCHEMIC HEART DISEASE IN THE GENERAL POPULATION?

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HDL cholesterol (HDL-C) is a major risk factor for ischemic heart disease (IHD). We have recently shown that both rare (mutations) and common (SNPs=single nucleotide polymorphisms) genetic variation in *ABC transporter A1 (ABCA1)* contributes to HDL-C in the general population. Whether genetic variation in *ABCA1* also predicts risk of IHD in the general population is not known. We determined the predictive value for IHD of all nonsynonymous SNPs previously identified by resequencing *ABCA1* in about 200 individuals, and of a relatively common mutation (frequency 0.4%) in 9,259 individuals from the general population during 25 years of follow-up. Five nonsynonymous SNPs predicted increased risk of IHD in genders combined (three SNPs), in women only (one SNP), or in men (one SNP). Hazard ratios varied from 1.2 to 1.7 for a single SNP, but increased up to 3.5 for pairwise combinations of SNPs. For the *ABCA1* mutation, incidence rates in non-carriers and heterozygotes were 61 and 157 per 10,000 person-years, respectively, corresponding to a hazard ratio of 2.4 for heterozygotes versus non-carriers. By the age of 80 years, about 48% of heterozygotes and 23% of non-carriers had IHD. These results were verified in a large, independent case-control study. In conclusion, we show that both rare and common genetic variation in *ABCA1* predicts risk of IHD in the general population, and that pairwise combinations of SNPs identify subgroups of individuals at substantially increased risk.

EFFECTS OF SECOND GENERATION ANTIPSYCHOTIC DRUGS ON TRIGLYCERIDES, HDL, AND RELATED LIPIDS IN PATIENTS WITH SCHIZOPHRENIA

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Second generation (atypical) antipsychotic drugs are associated with increased diabetes risk, and some of these drugs (clozapine and olanzapine) have been reported to produce significant increases in triglycerides and cholesterol. Much of the previous data has been based on retrospective chart reviews or data based epidemiological surveys. To more accurately evaluate this risk, we have conducted a large cross-sectional study of chronic schizophrenic patients treated with single olanzapine, clozapine, risperidone, olanzapine and conventional antipsychotics and are conducting a 5 months random assignment study of the effects of olanzapine vs. risperidone. In the cross sectional study fasting levels of triglycerides were higher in clozapine and olanzapine than risperidone patients, but there was no difference in HDL, LDL, cholesterol or HDL/LDL ratios. Only clozapine patients had significantly higher triglyceride values than patients treated with older conventional antipsychotics. There was no significant difference among the four drugs in the percent of schizophrenic patients with a metabolic triad (triglycerides, HDL, LDL) of abnormal values characteristic of metabolic syndrome, although patients on clozapine and olanzapine had slightly non-significantly higher rates. In the prospective study there was no difference in fasting levels of triglycerides or HDL between patients treated up to 5 months with olanzapine vs. risperidone, and no difference between the two drugs in triglyceride, HDL, LDL or cholesterol responses to a fatty meal test. Our data results suggest that although clozapine and olanzapine may be associated with higher triglyceride levels than risperidone, these effects may not be persistent for olanzapine during longer term treatment and may, therefore, not contribute to a continuing differential risk.

RELATIONSHIP BETWEEN THE LEVELS OF ENDOTHELIAL PROGENITOR CELLS TO HDL-CHOLESTEROL IN PATIENTS WITH CEREBROVASCULAR DISEASE

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Objective: To investigate a possible association of HDL levels and circulating EPCs in a clinical study population. **Background:** Endothelial progenitor cells (EPCs) derived from bone marrow may improve endothelial function and are inversely correlated to cardiovascular risk, as calculated by Framingham risk score. The blood levels of EPCs may be reduced in patients with increased LDL cholesterol and increase with statin treatment. There are however no reports of a relationship of HDL cholesterol to EPC levels. **Methods:** We recruited 102 patients with stable cerebrovascular disease (symptoms of more than one month duration) and controls without any history of cerebrovascular disease. All patients had evaluation of vascular disease risk factors. The patients were divided into three groups based on their circulating EPC levels. Simple linear regression was used to see the effect of hdl on EPC colonies after controlling for statin. Multiple linear regression model was used to identify independent predictors of EPC level. **Results:** The mean age was 63.5 years (SD \pm 12.86). Of the 102 subjects in the sample, 65 (63.7%) were male and 37 (36.3%) were female. There were significant effect of HDL on EPC colonies after controlling for Statin (β = 8.642, p = 0.001). The number of EPC colony forming units were significantly lower in individuals with low HDL (below 1.08 ± 0.26 mM) compared with intermediate (1.28 ± 0.29 mM) and high HDL levels (1.57 ± 0.86 mM) (p =0.017) for those who were not on Statin. **Conclusion:** Our results indicate a very strong positive association between the circulating EPC levels and the blood levels of HDL cholesterol. This was significant only in patients who were not on statin therapy.

GENE REGULATION AT THE MOLECULAR LEVEL BY PPAR ALPHA AND GAMMA

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The peroxisome proliferator-activated receptors (PPARs) have been implicated to play an important role in obesity-related metabolic diseases such as dyslipoproteinemia, insulin resistance and coronary artery disease. PPAR subtypes alpha and gamma have distinct expression patterns and recent advances with micro array analyses and gene targeting studies have helped delineate the subtype-specific function and the therapeutic potential of these receptors. We have recently assessed the mode of action of the PPAR agonists in the regulation of TG and HDL metabolism. The mechanism of action occurs at the molecular level and involves the activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR alpha) in the liver and in macrophages. PPAR alpha agonists decrease TG plasma concentrations by increasing the expression of lipoprotein lipase and decreasing apolipoprotein C-III concentrations. Interestingly, we recently discovered a new apolipoprotein (apo AV). Apo AV gene has been shown in apo AV transgenic and knockout mice and human association studies to be important in determining plasma TG levels. We demonstrate that apo AV is a PPAR alpha target gene and supporting its role as a major mediator for how fibrates reduce plasma triglycerides in humans and mice. The main hypothesis for the protective effect of HDL against atherosclerosis is related to its key function in reverse cholesterol transport, a crucial process for body cholesterol balance. The recent discovery by our lab of the protective role of human apolipoprotein A-I containing particles (LpA-I, LpA-I:A-II) and the identification of novel HDL receptors (CLA-1 / SR-BI, ABC1) provide new avenues for treatment of atherosclerotic cardiovascular disease. Very recently, we have found that the expression of genes which encode the “cholesterol efflux regulatory protein” (CERP), is also up-regulated by PPAR alpha agonists.

ROLE OF REGIONAL FAT IN SPLANCHNIC AND SYSTEMIC FFA METABOLISM

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The association between the metabolic complications of obesity and visceral fat mass has led to the hypothesis that visceral adipose tissue lipolysis, by delivering large amounts of free fatty acids (FFA) into the circulation, directly impacts metabolic abnormalities. Increased FFA concentrations have been shown to produce insulin resistance with respect to glucose metabolism in muscle and liver, stimulate insulin secretion, result in abnormal vascular regulation, and increase VLDL triglyceride secretion. Because understanding the adipose depot source of excess FFA may inform treatment options we performed a series of studies to measure the lipolytic activity of leg, splanchnic and upper body non-splanchnic adipose tissue in humans. We found that, although visceral fat mass in positively correlated with adverse health consequences and excess FFA availability, visceral fat is not the source of excess systemic FFA availability. Upper body non-visceral fat contributes the majority of FFA in lean, obese, diabetic and non-diabetic humans. Increasing amount of visceral fat probably result in greater hepatic FFA delivery. Systemic, as opposed to hepatic, insulin resistance is unlikely to be caused by high rates of visceral adipose tissue lipolysis.

LINKS BETWEEN ADIPOSE TISSUE AND VLDL SECRETION

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The liver secretes triglyceride (TG) in the form of very-low-density lipoprotein (VLDL) particles. The fatty acids that form the VLDL-TG can arise from several sources: from plasma non-esterified fatty acids (NEFA) arising from adipose tissue, from hepatic uptake of lipoprotein-TG and from *de novo* lipogenesis. The last seems to be a relatively minor route in humans on typical western diets. In the fasting state, lipoprotein-TG uptake reflects recycling of hepatic particles. Therefore, at least in the fasting state, adipose tissue NEFA release determines the average rate of VLDL-TG secretion. This accounts for older isotopic studies showing strong relationships between plasma rates of appearance of NEFA and of TG. However, when examined in more detail and changing nutritional states, this relationship is not strong. Data from adipose tissue and hepatic venous catheterization studies show little correspondence in time between adipose tissue NEFA and hepatic TG release. Intrahepatic regulation of TG storage and mobilization, and fatty acid oxidation must be involved in acute regulation of TG secretion. In the postprandial period, isotopic tracer studies together with specific isolation of VLDL particles show that dietary fatty acids appear in VLDL-TG within about 2 h. The pathway appears to involve hydrolysis of chylomicron-TG in adipose tissue with delivery of both NEFA and remnant-TG to the liver. Again, therefore, adipose tissue is dictating the supply of fatty acids to the liver. Finally, adipose tissue is a site of clearance of VLDL-TG. VLDL-TG are not so avidly cleared as chylomicron-TG, but studies using isotopic labelling of VLDL-TG show significant removal in adipose tissue even in the postprandial period when there is competition for removal from the chylomicrons. In summary, there are intimate connections between adipose tissue metabolism, hepatic TG secretion and the concentrations of TG-rich lipoproteins in plasma. Adipose tissue could be a target for modulating TG concentrations. I wish to thank my many colleagues in Oxford and Copenhagen for their contributions to the studies described.

THE ROLE OF PPAR GAMMA IN FATTY LIVER AND VLDL SECRETION

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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 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). Although significant evidence supports roles for increased plasma FA flux to the liver and hepatic uptake of remnant lipoproteins in the regulation of VLDL secretion, less is known about the importance of lipogenesis. In mice, SREBP-1c, as a regulator of lipogenesis, 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. In a recently completed human study, pioglitazone treatment reduced VLDL TG levels in patients without diabetes. However, pioglitazone did not affect VLDL TG secretion by the liver; rather, treatment with pioglitazone was associated with increased lipolysis of VLDL TG. The latter was associated with increased plasma mass of lipoprotein lipase and reduced hepatic secretion of apoC-III. The basis for the inability of this PPAR γ agonist to lower VLDL TG secretion, despite a reduction in plasma FA levels and improved insulin sensitivity, is under investigation.

PATHOGENESIS AND ASSESSMENT OF CARDIOVASCULAR RISK IN THE METABOLIC SYNDROME

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The metabolic syndrome is a cluster of abnormalities that lead to increased risk for both cardiovascular disease and type 2 diabetes mellitus. Major features used to define the syndrome include abdominal adiposity, atherogenic dyslipidemia (high triglyceride, low HDL, and small LDL particles), raised blood pressure, and abnormalities in glucose metabolism resulting from insulin resistance. Associated abnormalities include proinflammatory and prothrombotic states and hepatic steatosis. A number of studies have confirmed that the presence of metabolic syndrome confers significantly increased risk for coronary heart disease and stroke, although it is uncertain whether this risk exceeds that attributable to the sum of the independent effects of the individual components. Because of the predominance of small, dense, cholesterol-depleted LDL particles (LDL phenotype B) in the majority of patients with the metabolic syndrome, apoB, as a measure of the numbers of these particles, or direct measurements of LDL subclass concentrations, may be more indicative of cardiovascular risk than LDL cholesterol. ApoB level also includes the contribution of atherogenic triglyceride-rich lipoprotein remnant particles, as does the measurement of total cholesterol minus HDL cholesterol. Plasma CRP is also strongly and independently predictive of cardiovascular disease risk, although the pathophysiologic basis for this is not known. Weight loss achieved by reduced caloric intake and increased physical activity can reverse or ameliorate features of the metabolic syndrome in many patients. Persistence of the syndrome should lead to consideration of pharmacologic measures aimed at reducing overall cardiovascular disease risk, primarily by management of dyslipidemia and hypertension.

ATHEROGENIC DYSLIPIDEMIA: CHARACTERISTICS AND TREATMENT IN PATIENTS WITH DIABETES

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Type 2 diabetes mellitus (T2DM) is associated with a marked increase in risk for atherosclerotic cardiovascular disease (ASCVD), derived not simply from the concomitant hyperglycemia, but from the associated cluster of risk factors, including hypertension and dyslipidemia. The dyslipidemia is characterized by elevated blood levels of triglycerides (TG), low levels of high density lipoprotein (HDL) cholesterol, and average levels of low density lipoprotein (LDL) cholesterol carried on smaller and denser LDL particles. This trio of abnormalities is driven, in the main, by increased secretion of very low density lipoproteins (VLDL) carrying TG from the liver. Diet, exercise, and weight loss can impact significantly on the dyslipidemia, but typically pharmacologic approaches are required to reach "optimal" lipid levels. LDL cholesterol lowering should be the first goal, with targets of less than 100 mg/dl for all patients with T2DM and below 70 mg/dl for T2DM patients with existing ASCVD. Treatment with statins is the first approach, with the choice of titration to high doses or addition of bile acid sequestrants or plant stanols/sterols or ezetimibe in patients who do not get to goal on the initial dose of statin. Lowering of TG and raising of HDL are secondary targets, but should be considered as important targets in these patients. Fibrates and niacin have been successful in reducing ASCVD events in two large trials. The question of whether LDL should be treated to very low levels before addressing TG and HDL, or a more balanced attack on all three lipids should be undertaken cannot be answered at present. Additionally, the role of insulin sensitizers (PPAR γ agonists or dual PPAR γ /PPAR α agonists) as lipid altering agents is only partially understood at this time.

FUTURE OPTIONS IN THE MANAGEMENT OF DIABETIC PATIENTS WITH DYSLIPIDEMIA: ROLE OF THE DUAL ALPHA-GAMMA PPAR ACTIVATORS

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Pioglitazone and rosiglitazone are members of the thiazolidinedione class of drugs. They bind to the PPAR γ receptor, causing stem cells in subcutaneous fat depots to proliferate into mature adipocytes, which take up free fatty acids from the plasma compartment. The increase in subcutaneous fat mass is associated with a reciprocal decline in visceral fat content. The thiazolidinediones also inhibit lipolysis, contributing to the reduction in plasma FFA/intracellular toxic lipid metabolites and to improved insulin signaling. The resultant decline in plasma FFA leads to a decrease in HGP and increased muscle sensitivity to insulin. Thiazolidinediones also reduce hepatic fat content in association with increased splanchnic glucose uptake and enhanced suppression of HGP by insulin. In diabetic animal models, thiazolidinediones preserve beta cell function by reducing intracellular fatty acyl CoA levels. Beneficial effects of TZDs on insulin secretion also have been demonstrated in human patients with GDM and type 2 diabetes in the TRIPOD study and in the Diabetes Prevention Program. Most recently, thiazolidinediones have been shown to inhibit the release of resistin/IL-6/other adipocytokines and increase the release of adiponectin from fat cells. These changes in circulating adipocytokine levels are associated with improved hepatic and muscle sensitivity to insulin. Clinically, the glucose lowering effect of thiazolidinedione monotherapy is similar to that of metformin and the sulfonylureas. (Δ HbA_{1c} and Δ FPG = \sim 1.5% and 50-60 mg/dl, respectively). The thiazolidinediones also favorably impact a number of CAD risk factors and their use as anti-atherosclerotic agents currently is being investigated. Recently completed studies indicate that the dual (γ/α) PPAR activators (glitazars) are at least as efficacious as the currently available thiazolidinediones in reducing HbA_{1c} and more effective in reducing plasma triglyceride levels and elevating plasma HDL cholesterol levels.

MECHANISM OF PPAR ALPHA AND GAMMA ACTION IN THE MODULATION OF INFLAMMATORY PROCESS AND IN ATHEROSCLEROSIS

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A large body of data gathered over the past couple of years has identified the peroxisome proliferator-activated receptors (PPAR) α , γ , and β/δ as transcription factors exerting modulatory actions in vascular cells. PPARs, which belong to the nuclear receptor family of ligand-activated transcription factors, were originally described as gene regulators of various metabolic pathways. Although the PPAR α , γ , and β/δ subtypes are \pm 60% to 80% homologous in their ligand- and DNA-binding domains, significant differences in ligand and target gene specificities are observed. PPAR α is activated by polyunsaturated fatty acids and oxidized derivatives and by lipid-modifying drugs of the fibrate family, including fenofibrate or gemfibrozil. PPAR α controls expression of genes implicated in lipid metabolism. PPAR γ , in contrast, is a key regulator of glucose homeostasis and adipogenesis. Ligands of PPAR γ include naturally occurring FA derivatives, such as hydroxyoctadecadienoic acids (HODEs), prostaglandin derivatives such as 15-deoxy $\Delta^{12,14}$ -prostaglandin J₂, and glitazones, insulin-sensitizing drugs presently used to treat patients with type 2 diabetes. Ligands for PPAR β/δ are polyunsaturated fatty acids, prostaglandins, and synthetic compounds, some of which are presently in clinical development. PPAR β/δ stimulates fatty acid oxidation predominantly acting in muscle. All PPARs are expressed in vascular cells, where they exhibit antiinflammatory and antiatherogenic properties. In addition, studies in various animal models as well as clinical data suggest that PPAR α and PPAR γ activators can modulate atherogenesis *in vivo*. Given the widespread use of PPAR α and PPAR γ agonists in patients at high risk for cardiovascular disease, the understanding of their function in the vasculature is not only of basic interest but also has large clinical implications.

POSTPRANDIAL LIPOPROTEINS AND ENDOTHELIAL DYSFUNCTION. IN VITRO AND IN VIVO STUDIES

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The molecular mechanisms underlying the relationship between elevated plasma concentration of triglyceride-rich lipoproteins (TGRL) and coronary artery disease remain uncertain; evidence is accumulating to suggest that endothelial dysfunction is involved. To address this issue, we have first investigated the gene expression pattern and intracellular pathways in human endothelial cells incubated with TRGL, second we investigated whether changes in RLPs plasma levels during the postprandial phase relate to alterations of the endothelial function. A total of 53 subjects, 30 hypertriglyceridemic (TG levels 284.4 \pm 101.1 mg/dL) and 23 normotriglyceridemic (TG levels 108.65 \pm 39.9 mg/dL) were enrolled into the study. Human endothelial cells were incubated with TGRL isolated from hypertriglyceridemic (H-TGRL) and normotriglyceridemic (N-TGRL) subjects. Western blotting analysis and protein/DNA arrays showed that H-TGRL mainly activated p38MAPK, CREB and NF-kB. Total RNA was processed for cDNA microarray analysis. H-TGRL mainly induced the expression of adhesion molecules such as VCAM-1, PECAM-1, ELAM-1, P-selectin, chemotactic factors such as MCP-1, cytokines such as IL-6, receptors such as TLR-4 and CD40, and proteases such as PAI-1 and ADAMTs1. This profile was characteristic of H-TGRL as N-TGRL increased only the expression of VCAM-1, PECAM-1 and PAI-1. These findings were validated with quantitative real-time PCR and immunofluorescence studies. Moreover cholesterol in RLPs contributes significantly to the endothelial dysfunction occurring during the postprandial lipemia. These findings confirm the involvement of VLDL and Ox-VLDL in endothelial dysfunction and suggest new genes and molecular mechanisms involved in these actions.

PROMINENT APO B LIPOPROTEIN PARTICLE TYPES DURING POSTPRANDIAL LIPEMIA

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Postprandial hypertriglyceridemia contains a diverse group of apoB lipoproteins. Both intestinal (apoB48) and hepatic (apoB100) lipoproteins increase in response to a fatty meal. However, even with high fat intake, apoB100 comprises the majority of apoB lipoproteins as well as the increment over the fasting concentrations. Intestinal apo B lipoproteins are secreted over a wide range of sizes, from large VLDL-size to smaller LDL-size particles. Although much of the dietary triglyceride circulates with apoB48, the very low B48 concentrations, fasting or postprandial, compared to apoB100, suggest a minor role in atherogenesis. Both fasting and postprandial apoB lipoproteins contain triglyceride-rich remnant lipoproteins rich in apoC-III. In type 2 diabetic patients, a high fasting concentration of LDL particles that have apoCIII raised risk 7-fold for recurrent coronary events. ApoCIII is also a strong risk factor in nondiabetic people, independent of triglyceride and other risk factors. Lipoproteins with apoCIII are at higher concentrations in type 2 diabetes than those without these conditions, although this may be explained by high TG levels rather than insulin resistance. It is unclear whether increased postprandial response is a specific characteristic of CVD or diabetes, or simply a consequence of hypertriglyceridemia in general. Statins and fibrates reduce postprandial TG and remnant concentrations, apoB48 and apoB100, with or without apoCIII.

APO A-II IMPAIRS CHYLOMICRON CATABOLISM AND PROVOKES POSTPRANDIAL HYPERTRIGLYCERIDEMIA

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Postprandial hypertriglyceridemia and low plasma HDL levels are features of the metabolic syndrome and are also displayed by transgenic mice with moderate to high expression of human apolipoprotein A-II (hapo A-II). Hypertriglyceridemia in hapo A-II transgenic mice resulted from inhibition of lipoprotein lipase and hepatic lipase activities by hapo A-II carried on VLDL (Boisfer et al. J. Biol. Chem. 1999;274:11564-72).

This study aimed to determine whether the association of hapo A-II with triglyceride-rich lipoproteins (TRL) is sufficient to impair their catabolism.

To measure postprandial TRL residence time in plasma, intestinal TRL production was induced by intragastric administration of sunflower oil mixed with [¹⁴C]-triolein. [¹⁴C]-triglyceride (TG) was rapidly cleared in control mice but accumulated in plasma of transgenic mice, proportionately to hapo A-II concentration. Human apo A-II (synthesized in liver) was detected in chylomicrons (produced by intestine), indicating association of apo A-II with chylomicrons in plasma. This was confirmed by the absence of apo A-II in chylomicrons and VLDL of transgenic mice injected with Triton WR 1339, which prevents apolipoprotein exchanges.

These data show that hapo AII associates with TRL in the circulation thereby impairing their catabolism by LPL and provoking their prolonged accumulation. We have thus identified a novel mechanism of postprandial hypertriglyceridemia, which may be involved in some subjects with the metabolic syndrome.

ACCELERATED INTESTINAL ABSORPTION OF DIETARY LIPIDS CAUSES POSTPRANDIAL HYPERTRIGLYCERIDEMIA IN CD36 KNOCKOUT MICE

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Background: Human CD36 deficiency is a monogenic form of metabolic syndrome, we reported that they had dyslipidemia at fastig and postprandial states at the TG 2003. The aim of present study was investigating mechanisms underlying postprandial hypertriglyceridemia. **Methods and Results:** The animals used were CD36 knockout and wildtype mice. Oral fat loading test (OFLT) were performed using olive oil, showing that the peak and area under curves of TG were much greater in CD36 knockout mice, which were similar to those observed in human CD36 deficiency. And we have performed the following combined experiments. First, we injected Triton WR 1339 at fasting state without OFLT. We found no difference of serum TG levels between the two groups. It implied there was no change in the hepatic TG secretion. To the contrary, when we injected Triton WR 1339 and then performed OFLT, higher serum TG levels were observed in CD36 knockout mice, suggesting that there was an accelerated intestinal secretion of lipoproteins. In order to ascertain this, we analyzed lipids and lipoproteins of intestinal lymph fluid by puncturing cisterna chli after OFL. We found higher TG levels in lymph fluid of CD36 knockout mice. Finally, in the histological analysis by Oil red O staining of sections of small intestines, oil droplets were observed much abundant in the earlier phase of OFL.

Conclusions: These results suggest that the accelerated fat absorption from small intestine is the cause of postprandial hypertriglyceridemia in CD36 deficiency.

POSTPRANDIAL BUT NOT POSTABSORPTIVE LOW-DENSITY LIPOPROTEINS UPREGULATE THE EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 IN HUMAN AORTIC ENDOTHELIAL CELLS

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Clinical Division of General Internal Medicine, Clinical Department of Internal Medicine, Innsbruck Medical University, Innsbruck, Austria. The magnitude of postprandial lipemia has been identified as independent risk factor for the development of coronary artery disease. One of the first steps in atherogenesis is the adherence of leukocytes to the endothelium mediated by adhesion molecules. We tested the hypothesis that postprandial low-density lipoproteins (LDL) may be more effective than postabsorptive LDL in upregulating adhesion molecules on endothelial cells. LDL were isolated from human plasma before and 4 hours after ingestion of a fatty test meal. We used flow cytometry and Northern blotting to quantify cell adhesion molecules in human aortic endothelial cells. The adherence of leukocytes to endothelial cells was analyzed using a monocyte adhesion assay. Incubation of endothelial cells with postprandial, but not postabsorptive LDL induced a twofold increase of intercellular adhesion molecule-1 (ICAM-1) surface expression. No differences were observed for E-selectin and vascular cell adhesion molecule-1. In addition, increased amounts of ICAM-1 transcripts were found in endothelial cells treated with postprandial LDL. The adhesion of monocytes to endothelial cells was increased after pretreatment with postprandial, but not with postabsorptive LDL. We conclude that postprandial, but not postabsorptive LDL increase the surface expression of ICAM-1 in human aortic endothelial cells. The increase of ICAM-1 appears to be due to *de novo* protein synthesis and leads to increased adhesion of monocytes. The upregulation of ICAM-1 by postprandial LDL may explain part of the proatherogenic effect of high postprandial lipemia.

ELEVATED REMNANT-LIKE PARTICLES CHOLESTEROL LEVELS (RLP-C) IN FAMILIAL COMBINED HYPERLIPIDEMIA

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Objective: FCH is characterised by elevated levels of total cholesterol, triglycerides and apolipoprotein B and associated with an increased risk of CVD. High RLP-C levels are an independent risk factor for CVD. We investigated whether patients with FCH have high plasma RLP-C levels and if so, whether RLP-C levels contribute to the increased risk of CVD.

Methods: In total 582 subjects, including 134 FCH patients, 387 normo-lipidemic (NL) relatives and 61 spouses were studied. The diagnosis FCH was based on absolute apoB levels in combination with TG and TC levels using the nomogram (Vleuten et al Circulation 2004;109:2980-5). The RLP-C concentration was determined using immuneseperation technique.

Results: For both men and women, the mean plasma RLP-C concentration (mmol/l) was severely elevated in FCH patients (0.59 (0.54-0.66) and 0.40 (0.37-0.43), respectively) compared to both NL relatives (0.27 (0.26-0.29) in male and 0.22 (0.21-0.23) in female, $p < 0.000$) and spouses (0.27 (0.23-0.31) in male and 0.24 (0.21-0.27) in female, $p < 0.000$). Plasma RLP-C levels above the 90th percentile were associated with older age, increased WHR, and an atherogenic lipoprotein profile i.e. high TG levels, low HDL cholesterol, high non-HDL-c and more small, dense LDL. Gender, non-HDL-c, and triglycerides were the main predictors of plasma RLP-C explaining 72% of its variation. The 90th percentile of the plasma RLP-C concentration was associated with an increased risk for CVD (OR 3.55 (1.97-6.39), even after correction for age and gender (OR 2.65 (1.33-5.29) and independent of triglyceride levels (OR 2.35 (1.15-4.83)). However, this association was not independent of non-HDL-c levels.

Conclusion: Patients with FCH have increased RLP-C levels which contribute to the atherogenic lipoprotein profile and the increased risk for CVD.

POSTPRANDIAL LIPEMIA INDUCES INSULIN RESISTANCE INDEPENDENTLY OF NON-ESTERIFIED FATTY ACID PLASMA LEVELS

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Background The association between insulin resistance and pronounced postprandial lipemia is well established but any cause/effect relationship is poorly understood. In contrast to the general view that pronounced postprandial lipemia is a consequence of insulin resistance we tested the hypothesis that the phase of postprandial lipemia induces a state of temporary insulin resistance.

Methods A crossover design was performed involving ten healthy male volunteers to calculate insulin sensitivity both in the postabsorptive and postprandial state, i.e. three hours after ingestion of a standardized meal. Two virtually isocaloric meals were designed to produce a rise in triglycerides but a sharp contrast in the resulting non-esterified fatty acid (NEFA) levels, with meal 1 giving a rise to 169 percent ($P < 0.001$) and meal 2 a drop to 35 percent ($P = 0.02$) of postabsorptive NEFA levels. Both meals were given to each subject on two occasions. Insulin sensitivity was calculated using two methods, i.e. a frequently sampled intravenous glucose tolerance test with minimal model analysis and the reciprocal value of HOMA-IR, i.e. $1/\text{HOMA-IR}$.

Results Compared to the postabsorptive state, insulin sensitivity after each meal was impaired to a comparable degree irrespective of the sharply contrasting postprandial NEFA levels. With minimal model analysis, insulin sensitivity decreased by 36 percent after meal 1 ($P < 0.001$) and by 46 percent after meal 2 ($P = 0.02$). Using $1/\text{HOMA-IR}$, insulin sensitivity decreased by 30 percent after meal 1 ($P = 0.02$) and by 53 percent after meal 2 ($P = 0.003$).

Conclusions The postprandial rise of triglycerides induces a state of insulin resistance independently of postprandial plasma NEFA levels.

GREATER INCREASE IN POSTPRANDIAL SERUM TRIGLYCERIDES AND OXIDATIVE STRESS AFTER CONSUMPTION OF FISH OIL IN COMPARISON TO SOY OR OLIVE OIL IN MICE: POSSIBLE ROLE FOR PARAOXONASE 1 (PON1) TRIGLYCERIDE LIPASE-LIKE ACTIVITY

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Increased postprandial (PP) concentrations of triglyceride-rich lipoproteins are positively correlated with coronary heart disease risk, and the type of fatty acids (FA) consumed in a meal has been shown to influence the subsequent PP triglyceride response. We hypothesize that the composition of various FA may influence the subsequent PP triglyceride concentration and serum oxidative stress response. Thirty six Balb/C control mice were randomly divided into 4 groups. Each group was administered by gavage 300 μL of water (Control), fish (n-3 FA), soy (n-6 FA) or olive (n-9 FA) oils and blood samples were drawn after 2 hours. PP serum triglyceride level increased significantly after consumption of all oils, with the most significant increase (by 2.4 fold) observed after fish oil consumption. In parallel, serum lipid peroxidation increased by 3 fold after fish oil consumption, and this effect was accompanied by a 24% reduction in serum paraoxonase 1 (PON1) activity. We next questioned whether PON1 can affect PP triglycerides and serum oxidative stress. After administration of fish oil, PP serum triglyceride level and oxidative stress increased by 5 and 4 fold, respectively, in PON1 transgenic mice, in comparison to only 2 and 1.8 fold increase, respectively, in PON1 knockout mice. These results suggest that PON1 is involved in triglyceride absorption. In support to this involvement, PON1 triglyceride lipase-like activity was observed upon incubation of PON1 with triglyceride-rich particles.

METABOLIC BENEFITS OF COMBINED DIETARY INTERVENTIONS: DIETARY PORTFOLIO

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Lipid-lowering medications and diet have been shown independently to be effective in reducing cardiovascular disease risk and mortality. The apparent ineffectiveness of conventional 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 and the American Heart Association now recommend the use of functional foods or foods high in components which reduce cholesterol as options in the dietary strategy. These functional ingredients include viscous fibers, soy protein, plant sterols and nuts. Furthermore these four foods or food components have all been granted the right by the US Food and Drug Administration to make a health claim that they reduce the risk of cardiovascular disease. Individually they have been shown to lower serum cholesterol by 4-10%. To determine the extent to which a combination of cholesterol-lowering foods (dietary portfolio) could reduce serum cholesterol short-term (one month), metabolically controlled studies were carried out in which the dietary portfolio was compared with the results of metabolic studies in the same group of hypercholesterolemic subjects on NCEP step 2 diets with or without a statin (20 mg lovastatin). Thirty percent reductions in LDL-C were seen on the dietary portfolio, less than the statin at 34% LDL-C reduction but still clinically meaningful. However, the question remained as to whether this combination dietary approach has any application in the 'real world'. Current data suggest that approximately one third of individuals can maintain LDL-C reductions of 20% or more under real world conditions at 6 months.

NON-STATIN APPROACHES TO TREATING TG/HDL

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Seven treatments beyond statins exist for triglyceride and HDL. Treatment combinations yield best results. (1). **Diet:** In simple hypercholesterolemia (HC), carbohydrate intake should be <60en%, >25% fat to avoid induction of HTG. Persons with combined hyperlipidemia (CHL) already are TG induced at average macronutrient intakes, but TG falls at >40en% fat, <45en% carbohydrate intakes. Intake of allowable fats above current NCEP Guidelines holds promise for TG management. (2). **Fish Oil:** Two fish meals/week (= to ~ 350 mg/day omega-3) yield CVD benefit, but 3-9 g/d of omega-3 are needed for ~50% TG lowering, now feasible with a 90% formulation. (3). **Niacin:** Up to 3g/d plain or 2g/d time release niacins lower TG and raise HDL-C, both ~ 30%. LDL consistently converts to the large buoyant form. Flushing symptoms are reduced ~50% with aspirin and ingestion with food and no hot liquids. Hepatotoxicity with anorexia, flu-like symptoms, subtle AST/ALT rises and marked decreases in LDL and HDL-C improves with discontinuation. (4). **Fibric Acids** are short chain fatty acids, eg: chlorophenoxyisobutyric acid = clofibrate. Like fatty acids, fibrates activate PPAR alpha nuclear receptors, induce fatty acid oxidation, reduce TG synthesis, activate LPL mediated TG removal, stimulate apo A-I and decrease C-III secretion. TG falls ~50%, and HDL rises 10-15%, mainly as HDL₂-C. Hepatotoxicity is rare. Fibrate myotoxicity with statins occurs with gemfibrozil by inhibiting statin glucuronidation. Fibrates alone reduce CAD; efficacy with statins awaits the ACCORD Study. (5). **Cholesterol Absorption Inhibitors** bind to the gut NPC1L1 cholesterol transporter, reduce TG 8-11% and raise HDL-C 1-3%, possibly by decreased cholesterol delivery to the liver. (6). **Bile Acid Binding Resins** raise HDL-C ~ 3% and reduce CAD, additional to LDL lowering. (7). **CETP Inhibitors** bind to CETP, raise HDL-C ~50% and lower TG and LDL ~10%. CVD is reduced in animals, (?) in man.

IMPROVEMENT OF ENDOTHELIUM-DEPENDENT VASODILATION BY SIMVASTATIN IS FACILITATED BY COMBINATION WITH L-ARGININE IN PATIENTS WITH ELEVATED ADMA LEVELS

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Statins stimulate the expression of endothelial NO synthase (eNOS) in vitro and enhance endothelium-dependent, NO-mediated vasodilation in vivo. Asymmetrical dimethylarginine (ADMA) is an endogenous, competitive inhibitor of eNOS. Elevated plasma ADMA levels are associated with endothelial dysfunction. We investigated the hypothesis that simvastatin may enhance endothelial function in patients with elevated ADMA levels only if the inhibitory effect of ADMA is overcome by supplemental L-arginine, which serves as the natural substrate for NO synthase. Therefore we conducted a three period crossover trial including 15 asymptomatic, elderly subjects with elevated ADMA levels who received simvastatin (40 mg/day), L-arginine (3 g/day), or a combination of both in a randomized order. Each treatment was given for three weeks with a wash-out period in between. We assessed endothelium-dependent vasodilation by brachial artery ultrasound and determined ADMA and L-arginine plasma concentrations.

Simvastatin had no effect on endothelium-dependent vasodilation when administered alone (6.2±1.2% vs. 6.1±0.9%). L-arginine significantly improved endothelial function when given alone (8.7±0.7% vs. 4.9±0.8%; p<0.02) or in combination with simvastatin (9.8±1.5% vs. 5.3±0.8%; p<0.01). The effect of the combination was significantly greater than the mono-therapy (p=0.048). L-arginine significantly improved plasma L-arginine/ADMA ratio.

We conclude that simvastatin does not enhance endothelial function in subjects in whom eNOS activity is reduced by elevated ADMA levels. The combination of simvastatin and L-arginine improves endothelial function in these subjects.

EFFECTS OF CONCENTRIC AND ECCENTRIC MUSCLE EXERCISE ON LIPID AND GLUCOSE METABOLISM IN HEALTHY SEDENTARY INDIVIDUALS

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Background: Skeletal muscle can be exercised by two ways: Concentric muscle contraction is defined as active shortening of muscles, e.g. by stepping upwards, whereas eccentric muscle contraction is defined as active resistance to stretching, e.g. by stepping downwards. There are no data on the specific metabolic effects of concentric versus those of eccentric muscle work. **Methods:** Forty-five healthy sedentary volunteers were allocated randomly to two groups, one beginning with 2 months of concentric, the other with 2 months of eccentric exercise, followed by a cross-over for further 2 months. Patients were advised to exercise from 3 to 5 times a week. One exercise unit comprised a steady upward/downward hike over a difference in altitude of 510 meters, and lasted for about one hour. For the way back, a cable car was used. At baseline and after each exercise period a full metabolic profile including an oral fat tolerance test and an oral glucose tolerance test was obtained. **Results:** Compared to baseline the area under the glucose curve was improved significantly by 8.2% (p = 0.027) along with eccentric muscle exercise, but not along with concentric exercise (p = 0.145). The area under the triglyceride curve was significantly lowered only along with concentric exercise (by 11.0%; p = 0.037), but not with eccentric exercise (p = 0.567). LDL cholesterol was reduced significantly both along with concentric (by 10.2%; p <0.001) and eccentric exercise (8.9%; p = 0.001). **Conclusions:** Both concentric and eccentric muscle training have favourable effects on both lipid and glucose metabolism. Eccentric, but not concentric muscle training significantly improves glucose tolerance. Because many diabetic individuals are not able to perform concentric muscle exercise, eccentric muscle exercise should be tested as an exercise modality for diabetic patients.

MD-0727 IS A MINIMALLY ABSORBED INTESTINAL CHOLESTEROL ABSORPTION INHIBITOR

Mark Currie, Stephen Antonelli, Tim Barden, Wilmin Bartolini, Alex Bryant, Robert Busby, Brian Cali, Yueh-tyng Chien, Etschell Cordero, Chris Graul, Susan Hill, Peter Lee, Todd Milne, Regina Lundrigan-Soucy, Eduardo Martinez, Jim Pearson, Wayne Schairer, Kristie Sykes, John Talley, Tracy Weidert, Jing-Jing Yang, and Daniel Zimmer. Microbia Inc., Cambridge, MA 02141

Cholesterol absorption inhibitors (CAIs) are an important new class of lipid-lowering agents that work alone and in combination with statins, as the clinical success of ezetimibe and ezetimibe/simvastatin attests. We describe here a novel CAI clinical development candidate, MD-0727, with several important differentiating attributes in preclinical models: (1) very low absolute oral bioavailability, (2) potent inhibition of intestinal cholesterol uptake in both acute and chronic dosing studies, and (3) specificity of effect on cholesterol uptake. MD-0727 exhibits an absolute oral bioavailability in the rat, hamster, and dog of < 0.20, < 0.05 and 0.35%, respectively. MD-0727 inhibits cholesterol absorption in acute cholesterol uptake assays in the rat, hamster and mouse with an ED₅₀ of 0.008, 0.005, and 0.3 mpk, respectively. In dual plasma isotope cholesterol absorption studies in the rat, MD-0727 reduces cholesterol absorption by 80%. In a cholesterol-fed hamster model, treatment with MD-0727 once per day for 7 days results in >90% reduction in dietary-induced liver cholesterol accumulation (ED₅₀ = 0.02 mpk). MD-0727 does not alter intestinal absorption of taurocholate, progesterone, or retinal. Efficacy of ezetimibe and MD-0727 are equivalent, and they show no additivity, suggesting they act via the same pathway. The low systemic exposure and potency/efficacy profile of MD-0727 suggest it could be an important new stand-alone and combination agent for treatment of dyslipidemia. Phase I studies for MD-0727 are planned for the second half of 2005.

COMBINED THERAPY WITH OMEGA-3 FATTY ACIDS AND NIACIN IN ATHEROGENIC DYSLIPIDEMIA

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Niacin and omega-3 fatty acids (ω3 FA) are both nutrients that, in high doses, reduce serum triglycerides, and in the case of niacin, can raise HDL cholesterol (C) levels. The possibility that these two agents given in combination might have additive effects on these lipid parameters in patients with atherogenic dyslipidemia has not been previously examined. Accordingly, we conducted a series of studies in 28 patients with atherogenic dyslipidemia, half of which had diabetes mellitus (DM). All patients were randomly assigned to either dual placebo (n=7 non-DM and n=7 DM) or to a combination of 3.4 g/d of ω3 FA plus 3 g/d of crystalline niacin (n=7 non-DM and n=7 DM) for 12 weeks. Baseline and end-of-treatment lipid and lipoprotein assessments were made. No significant changes in any lipid parameter were observed in the placebo groups. Compared to baseline, combination therapy in the non-DM group reduced serum triglycerides by 52% (265±133 to 128±72, p=0.007), raised HDL-C by 34% (37±8 to 49±10, p=0.002), and had no significant effect on LDL-C (110±25 to 121±23, p=0.27). In the DM group, triglycerides fell by 51% (216±44 to 105±28, p=0.0005), HDL-C increased by 52% (42±7 to 63±26, p=0.047), and LDL-C fell by 13% (107±33 to 93±35, p=0.024). Neither plasma glucose (170±45 to 189±45 mg/dL, p=0.42) nor HbA1c (8.5±1.5 to 8.9±2.0, p=0.25) changed significantly in the DM patients. We conclude that combined therapy with niacin and ω3 FA has markedly beneficial effects on triglyceride and HDL-C levels in patients with atherogenic dyslipidemia regardless of diabetic status, and that the combination does not adversely affect glycemic control in DM patients.

MANAGEMENT OF METABOLIC SYNDROME: BEYOND CHOLESTEROL LOWERING

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Metabolic syndrome is associated with an increased incidence of atherosclerotic vascular disease and type II diabetes. The connection between heightened state of inflammation and vascular disease in metabolic syndrome is now accepted. The role of adipose tissue as a key source of mediators of atherosclerosis has only been recognized recently. The systemic increases in inflammatory and thrombotic factors including CRP, interleukin-6, TNFα, MCP-1, resistin and PAI-1 are directly linked to increased adipose tissue mass and infiltration of adipose tissue with activated macrophages. Accumulating evidence also suggests that insulin resistance in metabolic syndrome and diabetes causes endothelial dysfunction, impairing its anti-atherogenic role. Such endothelial dysfunctions include reduced endothelium-dependent vasodilation, enhanced leukocyte-endothelial cell interactions and the altered production of a variety of vasoactive substances that affect coagulation, extracellular matrix deposition and vascular smooth muscle physiology.

Management of metabolic syndrome focuses on reducing the underlying risk factors and emphasizes lifestyle modifications as first-line therapy including weight reduction, exercise and diet. Such life-style modifications can diminish the pro-inflammatory state as assessed by C-reactive protein. Bariatric surgery has a useful role in the management of morbid obesity although longer-term follow-up is needed. Pharmacological therapy of atherogenic dyslipidemia in clinical trials with statins or fibrates improved cardiovascular outcome and reduced the pro-inflammatory state. The benefits of blood pressure control for cardiovascular diseases has been demonstrated in many clinical trials although debate continues whether ACE inhibitors have a unique role as initial therapy in patients with type 2 diabetes. Aspirin is indicated as effective therapy of the pro-thrombotic state. While thiazolidinediones can reduce the pro-inflammatory state their use is not recommended specifically for this purpose at this time.

ONCE-DAILY EXTENDED-RELEASE NIACIN/LOVASTATIN COMBINATION IMPROVES CHOLESTEROL AND APOLIPOPROTEIN RATIOS COMPARED WITH ATORVASTATIN AND SIMVASTATIN

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As elevated TG/low HDL-C are important CHD risk factors, ratios of total cholesterol to HDL-C (TC/HDL-C) and apolipoprotein B to AI (apoB/AI) are potent predictors of myocardial infarction (MI) risk. We compared effects of a once-daily fixed dose extended-release niacin/lovastatin (ERN/L) combination versus atorvastatin and simvastatin. 315 patients were randomized in an open-label, 16 week titration study to ERN/L 1000/40 (mg niacin/mg lovastatin) or 2000/40, atorvastatin 10-40 mg, or simvastatin 10-40 mg. Mean age was 53 years, 72% were men, 20% had heart disease. Baseline values: median TG 170 mg/dL; mean HDL-C 38 mg/dL; mean TC/HDL 7.0 and apoB/AI 1.14.

Effects of ERN/L, Atorvastatin, and Simvastatin on Mean Ratios

Week 8	ERN/L 1000/40 mg	Atorva 10 mg	Simva 10 mg
apoB/AI	0.69 (-38%)*	0.83 (-30%)**	0.84 (-26%)
TC/HDL	4.1 (-39%)*	5.0 (-31%)**	5.1 (-25%)
Week 12	ERN/L 1000/40 mg	Atorva 20 mg	Simva 20 mg
apoB/AI	0.67 (-40%)*†	0.75 (-37%)**	0.77 (-32%)
TC/HDL	4.0 (-42%)*	4.6 (-37%)**	4.8 (-30%)
Week 16	ERN/L 2000/40 mg	Atorva 40 mg	Simva 40 mg
apoB/AI	0.62 (-45%)**	0.69 (-41%)**	0.74 (-35%)
TC/HDL	3.7 (-47%)*	4.2 (-42%)**	4.6 (-33%)

*p<0.05 vs. atorvastatin and simvastatin; **p<0.05 vs. simvastatin; †p=0.058 vs. atorvastatin

SAFETY CONSIDERATIONS FOR COMBINATION THERAPY FOR PATIENTS WITH METABOLIC SYNDROME

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The complementary effects of HMG-CoA reductase inhibitors (statins) and fibric acid derivatives (fibrates) have led to increasing use of statin/fibrate combination therapy, particularly for those patients with mixed dyslipidemia. Clinical experience indicates that there may be an increased risk of myotoxicity associated with fibrate/statin combination therapy. However, it is not known whether there are differences in the rate of myotoxicity between the use of fenofibrate and gemfibrozil in combination with statins. Gemfibrozil inhibits the glucuronidation and CYP 2C8 metabolic pathway resulting in higher statin blood levels as well as a number of drugs to treat diabetes. Fenofibrate does not interfere with statin metabolism and, therefore, may be a safer option to combine with a statin. To evaluate this, data from the Food and Drug Administration's Adverse Event Reporting System was reviewed to determine how many adverse events were reported for patients treated concomitantly with fibrates and statins. Using these data, the number of reported cases of rhabdomyolysis per million prescriptions dispensed was calculated. Differences were found in the reporting rate of rhabdomyolysis for fenofibrate and gemfibrozil. When used in combination with cerivastatin, the number of cases of rhabdomyolysis reported per million prescriptions dispensed was approximately 33-times lower for fenofibrate than for gemfibrozil. When used in combination with statins other than cerivastatin, the number of cases of rhabdomyolysis reported per million prescriptions dispensed was approximately 15-times lower for fenofibrate than for gemfibrozil. These findings suggest that the use of fenofibrate in combination with statins results in fewer reported cases of rhabdomyolysis per million prescriptions dispensed than the use of gemfibrozil. Therefore, when considering concomitant use of a fibrate and a statin, these data suggest that it may be safer to use fenofibrate rather than gemfibrozil.

WEIGHT LOSS INDEPENDENT EFFECT OF RIMONABANT ON HDL-C AND TRIGLYCERIDE LEVELS IN A 2-YEAR RANDOMIZED CONTROLLED TRIAL

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Rimonabant, the first selective cannabinoid-1 blocker, has been shown to produce significant weight loss and waist circumference reduction and improvements in HDL-C and triglyceride (TG) levels, of which about 50% was not attributable to the effect of weight loss. The present analysis evaluated further the pharmacologic effect of rimonabant on serum lipids using results of the RIO North America trial, a multicenter, randomized, placebo-controlled study in overweight or obese patients. After 1 year of treatment with placebo or rimonabant plus diet patients (n=1561) under active treatment were re-randomized either to the same rimonabant dose (5 mg: R5 or 20 mg: R20) or placebo (PLA) during year 2. Weight lost during year 1 was well maintained in patients who received rimonabant 20 mg (R20/R20) in year 2, while those re-randomized to placebo (R20/PLA) had significant weight regain (intent-to-treat, last-observation carried forward mean±SD 5.6±5.3 kg; P<0.001 vs R20/R20). Waist circumference also increased during year 2 in R20/PLA vs R20/R20 (+4.5 vs 0.7 cm; P<0.001). R20 produced a 12.6% increase in HDL-C during year 1 (P<0.001 PLA). HDL-C declined by 0.5% (-0.02 mmol/L) in year 2 in R20/PLA but increased by 5.8% (0.07 mmol/L) in R20/R20 (P<0.001). TG increased by 0.26 mmol/L in year 2 in R20/PLA but did not change in the R20/R20 group. TG increased slightly in R5/PLA but decreased in R5/R5 during year 2 (P=0.032). Thus, even in the absence of further weight loss during year 2, continued R20 treatment increased HDL-C further and sustained the reduction in TG levels achieved during year 1. The safety profile over a period of 2 years was good. These results provide further confirmation of a significant impact of rimonabant on atherogenic dyslipidemia which is independent of weight loss and support the long-term use of rimonabant in lipid management in overweight or obese patients.

LIPID-INDEPENDENT EFFECTS OF STATINS ON ENDOTHELIAL FUNCTION AND LEFT VENTRICULAR MASS IN HYPERTENSIVE-TREATED PATIENTS WITH METABOLIC SYNDROME

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Experimental evidence suggests a lipid-independent effect of statins on endothelial function and miocyte hypertrophy. This is a randomized, double-blind, placebo controlled study of one-year treatment with fluvastatin. Thirty-nine hypertensive-treated patients with metabolic syndrome and no prior CV events were given fluvastatin (F) or placebo (P). At randomization, they had same age (51±8 vs 51±9 years-old, F vs P, p=ns), triglyceride levels (187±187 vs 195±145 mg/dl, p=ns), HDL-cholesterol levels (51±12 vs 44±13 mg/dl, p=ns), left ventricular mass index (LVMI) (116±23 vs 100±37 g/m², p=ns) and same radial artery endothelial dependent diastolic diameter (EDDD) measured by ultrasound (153±264 vs 152±372 mm, p=ns). There was no difference on men/women proportion or dose/number of anti-hypertensive drugs taken between groups over the study period. Compared with placebo group (19 patients), fluvastatin therapy (20 patients) significantly reduced LVMI (100±23 vs 82±15 g/m², p=0,01) as well as EDDD (62±201 vs 385±549 mm, p=ns). No correlation between these parameters and lipoprotein levels (HDL, LDL or triglycerides) was observed during the study. Multiple linear regression analysis models was performed taking EDDD as dependent variable and systolic/diastolic BP, glucose levels, insulin levels, waist circumference, LVMI, age and fluvastatin therapy as independent variables. Only fluvastatin therapy predicted EDDD (P=0,02). Fluvastatin therapy in hypertensive-treated patients with metabolic syndrome resulted in additional reduction in LVMI and EDDD improvement. A lipid-independent effect of statins seems to be related with the observed results.

AHA-RECOMMENDED INTAKES OF OMEGA-3 FATTY ACIDS IMPROVE CARDIAC AUTONOMIC TONE BUT DO NOT REDUCE LIPIDS OR INFLAMMATORY MARKERS

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The cardioprotective effects of omega-3 fatty acids (ω-3 FA) have been well-documented. These FA are presumed to reduce susceptibility to fatal arrhythmias, but the mechanism by which this occurs is not known. The effects of AHA-recommended intakes of ω-3 FA on lipids, inflammatory cytokines, cardiac function, and arterial compliance have not been reported. Eighteen white males with documented CHD (age, 68±6.5 yrs; BMI, 30±4.4) and ejection fractions of <40% were randomized to either placebo or ω-3 FA (1.0 g EPA+DHA, Ocean-Nutrition Canada) for two, 4-month periods and then crossed over to the alternate treatment for another 4-months. At the end of each period, a non-invasive cardiovascular profile was obtained by radial artery tonometry (which included estimates of arterial compliance), heart rate (HR), blood pressure, and estimates of cardiac function. The rate of HR recovery post stress test was also assessed as were fasting serum levels of C-reactive protein, interleukin-6 and tumor necrosis factor-α. Supplementation with 1 g of ω-3 FA reduced resting pulse (73±13 to 68±13 bpm, p=0.001) and accelerated HR recovery 1-minute post exercise (n=14; -27±10 to -32±12 bpm, p<0.01). There was no significant effect on blood pressure, arterial compliance or inflammatory markers. Although AHA-recommended intakes of long-chain ω-3 FA did not affect classic or emerging CHD risk factors, they did lower HR and enhanced HR-recovery post exercise. These changes would be expected to reduce risk for sudden cardiac death.

EFFECT OF TREATMENT WITH SIMVASTATIN PLUS FENOFIBRATE ON TRIGLYCERIDE-RICH LIPOPROTEIN PROFILE IN PATIENTS WITH AND WITHOUT THE METABOLIC SYNDROME

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Patients with the metabolic syndrome (MS) often exhibit a dyslipidemia characterized by a predominance of triglyceride-rich lipoprotein (TGRLP) particles associated with a higher risk of CHD. Combination therapy with a statin+fenofibrate may improve TGRLP levels over treatment with statin alone. This double-blind, 18-week study compared % change from baseline to week 12 in TG and TGRLP levels in patients with combined hyperlipidemia (LDL-C >130 mg/dL and TG >150 mg/dL and <500 mg/dL) randomized to treatment with simvastatin 20 plus fenofibrate 160 mg (S20+F160) or simvastatin 20 mg (S20). We compared S20+F160 vs. S20 on various TGRLP subclasses measured by VAP (Vertical Auto Profile) technology in a predefined subset (n=372) of patients with combined hyperlipidemia with MS (>3 NCEP ATP III criteria) or without MS (n=151). In this population of patients, treatment with S20+F160 compared with S20 was more effective in reducing fasting TG levels and TGRLP, regardless of MS status (Table 1).

Table 1. Percent Change (SE) from Baseline in TG and TGRLP

Variable	Patients with MS (N=372)		Patients without MS (N=151)	
	S20 + F160	S20	S20 + F160	S20
Total TG	-47.0 (1.2)	-28.0 (2.1)	-50.2 (2.2)	-27.7 (2.8)
VLDL-C	-44.8 (1.4)	-24.5 (2.0)	-44.3 (2.2)	-22.0 (3.0)
VLDL ₁ -C	-47.2 (1.6)	-25.0 (2.3)	-46.2 (2.6)	-21.7 (3.6)
VLDL ₃ -C	-41.4 (1.2)	-23.2 (1.8)	-41.8 (2.0)	-21.4 (2.7)
VLDL _{3a} -C	-45.5 (1.5)	-23.5 (2.1)	-44.1 (2.3)	-23.4 (3.2)
VLDL _{3b} -C	-37.0 (1.1)	-22.2 (1.6)	-37.8 (1.8)	-20.3 (2.4)

*p<0.001 compared with baseline

ROLE OF PITAVASTATIN IN ARTERIAL CHOLESTEROL TRAFFICKING

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

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ALTERED DNA METHYLATION PATTERNS IN APOE NULL MICE: DIRECT EFFECTS OF PLASMA LIPIDS ON EPIGENETIC PARAMETERS?

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Epigenetics is the study of heritable variations in gene expression unrelated to DNA sequence, such DNA methylation. DNA methylation pattern is strongly associated with disease, in particular tumor growth, and is altered in vascular lesions and blood leukocytes during atherosclerosis. Genome-wide DNA hypomethylation in advanced atherosclerotic lesions may be explained by the atherosclerotic risk factor hyperhomocysteinemia. However, epigenetic alterations in atherosclerotic vessels may also be correlated to plasma lipid and lipoprotein levels. Our present data demonstrate that triglyceride-rich lipoproteins may even be causative in respect to epigenetic alterations.

ApoE null mice on normal diet were compared to wild type animals and demonstrated altered pattern of DNA methylation already at 4 weeks of age, where these mice are hyperlipidemic but lack signs of atherosclerotic development. In order to isolate the effect of lipoproteins, human THP-1 cells were incubated with lipoprotein mixtures representing plasma compositions of wild type or apoE null mice. Incubation of differentiated cells for only 24h in respective mixture resulted in significant DNA hypermethylation in the "apoE null" treated cells, indicating that lipoproteins have direct and strong effects on epigenetic pattern. Incubations with singular lipoproteins demonstrated that VLDL at this level (68.8 μ g/ml) has strong effect on DNA methylation pattern, whereas HDL or LDL lacked effect. In conclusion, hyperlipidemia appears to induce epigenetic changes in macrophages that may induce altered gene expression, and such epigenetic changes may be an early sign of atherosclerotic development, brought on by a pro-atherogenic lipoprotein profile.

ELECTRONEGATIVE TG-ENRICHED LDL AND VASCULAR INFLAMMATION

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Inflammatory mediators play a key role in the pathogenesis of atherosclerosis, and low-density lipoprotein (LDL) particles that have been oxidized in vitro have been shown to induce proinflammatory factors such as cell adhesion molecules, chemokines, and growth factors in endothelial cells. However, it is not clear whether circulating oxidized LDL has similar proinflammatory effects. LDL isolated from patients with hypercholesterolemia was fractionated by ion-exchange chromatography into 5 distinct subfractions, to examine the biological effects of the latest and most electronegatively charged fraction (F5) compared with those of the earliest fraction (F1) on cultured human umbilical vein endothelial cells (HUVEC). F5 is enriched in triglycerides, and incubation of HUVEC or aortic endothelial cells (AEC) with F5 led to increased adhesion of monocytes that could be blocked by antibodies to very late activation antigen-4 (VLA-4) or vascular cell adhesion molecule-1 (VCAM-1). Incubation of endothelial cells with F5 also increased production of the CXC chemokines growth-related oncogene (GRO)- α , GRO- β , GRO- γ , interleukin-8 (IL-8), epithelial neutrophil-activating peptide-78 (ENA-78), and granulocyte chemotactic protein-2 (GCP-2). The enzyme lipoprotein-associated phospholipase A₂ (Lp-PLA₂), which can generate lysophosphatidylcholine and oxidized fatty acids from oxidized LDL, is concentrated in F5.

APOLIPOPROTEIN C-III ISOFORMS: KINETICS AND RELATIVE IMPLICATION IN LIPOPROTEIN-LIPID METABOLISM.

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Apolipoprotein C-III (apoCIII) production rate (PR) appears as a key determinant of plasma TG levels in hypertriglyceridemic patients. ApoC-III is present in three different isoforms in the circulation according to its degree of sialylation. The present study investigated the kinetics and respective role of each apoC-III isoforms in modulating intravascular lipoprotein metabolism. Kinetic studies were performed in a sample of 18 healthy men (mean age \pm SD) 42.1 \pm 9.5 yrs, body mass index 29.8 \pm 4.6 kg/m²). Total plasma (d<1.25 g/ml) apoC-III kinetics were measured under constantly fed conditions using a primed-constant infusion of L-(5,5,5-D3) leucine for 12 hours. ApoC-III isoforms were separated by isoelectrofocusing and their plasma concentrations were determined by densitometry analysis. Isotopic enrichment of apoC-III over time was measured by GC-MS and kinetic parameters were obtained using multicompartmental modeling. Monosialylated (apoC-III₁) and disialylated (apoC-III₂) apoC-III exhibited similar kinetics, with PR (means \pm SD) of 1.22 \pm 0.49 mg/kg/d and 1.15 \pm 0.59 mg/kg/d respectively (P=0.24) and fractional catabolic rates (FCR) of 0.51 \pm 0.13 pool/d and 0.61 \pm 0.24 pool/d respectively (P=0.14). The PR (0.25 \pm 0.12 mg/kg/d) and FCR (0.21 \pm 0.07 pool/d) of non-sialylated apoC-III (apoC-III₀) were reduced by more than 60% (P<0.0001) compared to the other two isoforms. The PR of apoC-III₁ and apoC-III₂ were stronger correlates of plasma TG levels (r>0.8, P<0.0001) than apoC-III₀ PR (r=0.54, P>0.05). Of all three apoC-III isoforms, the various kinetic parameters of apoC-III₂ showed the strongest association (inverse) with features of the small dense LDL phenotype and of HDL particle size. These results suggest that the kinetics of all apoC-III isoforms are determinants of plasma TG levels in healthy overweight men, with an apparently greater contribution of the apoC-III₁ and apoC-III₂ isoforms. Our data also suggest that the kinetics of apoC-III₂ may be more specifically implicated in the expression of the small dense LDL phenotype as well as of small HDL.

EFFECT OF OXIDIZED HIGH DENSITY LIPOPROTEIN ON LYMPHOCYTE FUNCTION

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High Density Lipoprotein (HDL) possesses antiatherogenic properties. However, HDL can be efficiently oxidized *in vitro* and oxidized HDL is also found in atherosclerotic plaques. Lymphocytes are also present in atherosclerotic plaques, but it is not known if oxidized HDL influences lymphocyte function. Therefore, this study was undertaken to determine the influence of HDL oxidation on lymphocyte activity.

Peripheral mononuclear cells were isolated from 10 healthy males and incubated with autologous mildly oxidized HDL or native HDL (0 to 100 g/mL) in the presence of Concanavalin A for 12, 36 and 60 hours. Compared to control HDL, oxidized HDL significantly inhibited cellular activation, as measured by the expression of CD69. A decrease in the expression of IL-2 receptor and IL-2 concentration recovered in culture medium was also observed. Cells treated with oxidized HDL also showed a higher expression of CD11a. Oxidized HDL also significantly inhibited lymphocyte proliferation in a dose-dependant manner. Furthermore, the effects on CD69, IL-2 and CD11a were correlated with the amount of lipid peroxides present in the HDL.

We conclude that oxidized HDL can influence lymphocyte activity, and therefore could play a significant role in the inflammatory processes involved in atherosclerosis.

DECREASED LIPOPROTEIN LIPASE (LPL) ACTIVITY AND DELAYED POSTPRANDIAL CLEARANCE OF TRIGLYCERIDE-RICH LIPOPROTEINS (TRL) IN PERSONS WITH ECHOLUCENT CAROTID PLAQUES.

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Introduction: Persons with echolucent, lipid-rich atherosclerotic carotid artery plaques have increased risk of ischemic cerebrovascular events. A delayed chylomicron (CM) clearance has been identified as a risk factor for atherosclerosis, and prolonged postprandial triglyceridemia is associated with increased IMT in the carotid arteries. **Objective:** To determine the metabolism of postprandial triglycerides and LPL activity in relation to carotid plaque morphology. **Methods:** Plaque echogenicity of the carotid arteries was assessed by B-mode ultrasound and analysis of the grey scale median (GSM). Participants were recruited from a population health survey (57 persons with carotid plaques and 38 persons without carotid plaques). Echolucent plaques were defined as GSM at or below the median (≤ 65) and echogenic plaques as plaques with a GSM above the median (> 65). Blood samples were collected before and at 2-hours interval for 8 hours after a standard high fat meal. LPL activity was determined before and after heparin administration (100IU/kg). **Results:** A delayed clearance of chylomicron triglycerides from the circulation was observed in persons with echolucent plaques compared to controls ($p=0.04$). LPL activity was decreased in persons with echolucent plaques (112.1mU/ml, 94.3 – 133.1) (mean, 95% CI) compared to persons with echogenic plaques (137.5mU/ml, 119.9 – 157.8, $p=0.06$) and to controls (137.5mU/ml, 121.9 – 155.0, $p=0.04$). A linear increase in GSM across increasing tertiles of LPL was found (p for trend=0.02). **Conclusions:** The present study supports the hypothesis that TRL may be involved in the development of echolucent, lipid-rich plaques in the carotid artery. It further suggests that this may be due to lower LPL activity, and that low plasma LPL activity may promote fat accumulation in the arterial wall.

USING TRIGLYCERIDE LEVELS AT ESTABLISHED CUT-OFFS LEADS TO UNDER DIAGNOSIS OF INSULIN RESISTANCE IN NONHISPANIC BLACKS

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To clinicians the Metabolic Syndrome (MS) is evidence of insulin resistance. To diagnose MS 3 of 5 criteria must be met: $TG \geq 150$, low HDL, central obesity, hypertension and fasting hyperglycemia. Criteria to diagnose insulin resistance based only on lipids has also been developed. The lipid criteria (LC) are the presence of either: $TG \geq 130$ mg/dL or TG/HDL ratio ≥ 3 . The ability of the MS and the LC to identify insulin resistance has not been tested in a multiethnic sample. As nonhispanic blacks (NHB) often have insulin resistance yet normal TG levels, it is unknown if MS or LC will diagnose insulin resistance in NHB. In 5782 adults from National Health and Nutrition Examination Survey 1988-1994 the ability of the MS and the LC to diagnose insulin resistance in NHB, nonhispanic whites (NHW) and Mexican Americans were compared. Insulin resistance was assessed HOMA. The cohort was divided into tertiles of HOMA (≤ 1.57 , 1.58-2.49, ≥ 2.50). Insulin resistance was defined by the 3rd tertile. For NHB, NHW and MA TG levels were 99(2) mean(SE), 132(3), 139(3) resp. and TG/HDL levels were 2.1(0.1), 3.2(0.1), 3.3(0.1). TG and TG/HDL were lower in NHB than NHW or MA (all $P < 0.00001$). In diagnosing insulin resistance the sensitivities of the LC in the NHB, NHW and MA were 36%, 70%, 63% with specificities of 84%, 71%, 71%. For the MS the sensitivities were 42%, 59%, 53% with specificities of 89%, 81%, 88%. Hence in NHB the MS and LC were insensitive to the diagnosis of insulin resistance. Further, with increasing TG levels, HOMA rose in all groups. But over the full range of TG, HOMA was 0.65 units higher in NHB than NHW and 0.39 units higher in NHB than MA. Thus insulin resistance occurred at lower levels of TG in NHB than NHW and MA. In fact, 77.7% of the insulin resistant NHB had $TG < 150$ mg/dL compared to 46.1% of NHW and 50.9% of MA. In NHB as insulin resistance occurs even when TG levels are below established cut-offs, using either the LC or the MS will lead to under diagnosis and lost opportunity for disease prevention.

FATTY ACID PROFILES AND INTERACTIONS IN CIRCULATING LIPIDS IN DEFINING STRATEGIES FOR DIETARY STUDIES IN POPULATION GROUPS

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In our populations, fats provide 35-40 en% (> 90 g /day as fatty acids, FA). In contrast, the estimated load of circulating (esterified) FA in plasma amounts to 4-6g. Saturation of FA transport, competitions and displacements between FA and FA classes for incorporation into plasma lipids are therefore predictable. In addition, the chemistry of glycerolipids dictates the esterification processes of FA and FA classes: saturates (SFA) mainly in position *sn*-1 of glycerol, whereas monounsaturates (MUFA) and polyunsaturates (PUFA) mainly in position *sn*-2. FA analysis of plasma lipids and lipid classes (PL, TG, CE) in 75 subjects (66 M, 9 F, aged 48-76 y) on habitual diet, revealed that several (mainly negative) highly significant correlations between levels of FA and FA classes exist. The most relevant ones are: in total lipids, strongly negative between MUFA (oleic acid, 18:1 n-9) and n-6 PUFA (linoleic, 18:2, and arachidonic acid, 20:4), while no correlation is present between n-9 MUFA and n-3 PUFA, nor between n-6 and n-3 PUFA, SFA negative vs n-6 PUFA. In lipid classes, correlations among FA classes in PL and CE are similar to those in total lipids (mainly negative), whereas in TG mainly positive correlations are present. Precise and measurable processes control therefore the final incorporation and transport of FA in complex lipids. It appears that MUFA, i.e. oleic acid, displace n-6 PUFA but not n-3 PUFA, and n-6 and n-3 do not compete for plasma transport. The message to nutritionists is that high MUFA intakes would reduce circulating n-6 PUFA, but not n-3 PUFA, and that levels of n-3 PUFA are not affected by intakes of n-6, as also recently shown by epidemiological studies.

Finally, a simple, inexpensive and time-saving method developed in our lab for FA analysis in a drop of blood allows to overcome the limitations of conventional approaches in the applicability to studies on large or special (neonates) population groups.

CONTROL OF LIPID METABOLISM THROUGH TRANSCRIPTIONAL COACTIVATORS

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Many complex biological programs are controlled at the level of gene transcription by DNA-binding transcription factors. Recent studies have revealed a novel mode of regulation by coactivator proteins, best illustrated by the PGC-1 family of coactivators. These factors are highly responsive to a variety of environmental cues, from temperature to nutritional status to physical activity, and they coordinately regulate metabolic pathways and biological processes in a tissue-specific manner. For example, the expression of PGC-1 β in liver is highly inducible by dietary saturated fats, which lead to hyperlipidemia and atherogenesis. PGC-1 β coactivates the entire SREBP transcription factor family and stimulates lipogenic gene expression *in vivo*, but unlike SREBP itself, PGC-1 β reduces hepatic lipid accumulation while greatly increasing circulating triglycerides and cholesterol in VLDL particles. These data illustrate that PGC-1 β coordinates the programs of lipogenesis and lipid transport, and suggest a clear mechanism through which dietary saturated fats can stimulate hyperlipidemia and atherogenesis. These actions also raise new opportunities for the development of novel therapeutics.

RELATIONSHIP BETWEEN POSTPRANDIAL FREE FATTY ACIDS AND TRIGLYCERIDES IN NORMAL, DYSLIPIDEMIC AND TYPE 2 DIABETIC SUBJECTS

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The delivery of free fatty acids (FFA) to the liver is thought to be an important factor in the synthesis and secretion of very low density lipoprotein (VLDL) triglyceride (TG), a major determinant of circulating TG concentrations. However, previous studies have found no correlation between plasma FFA and plasma TGs. Relatively little attention has been given to postprandial FFA metabolism and its potential contribution to hepatic TG production. We measured nocturnal and postprandial FFA concentrations and palmitate rate of appearance (R_a) in control subjects with normal plasma TGs (C, N=11); nondiabetic dyslipidemic subjects receiving n-3 fatty acids (3 g/d), niacin (3 g/d), combined n-3 fatty acids and niacin, or placebo (DYS, N=28); and subjects with poorly controlled type 2 diabetes (DM, N=32). nocturnal and postprandial palmitate R_a (area under the curve analysis) were higher in DYS and DM than in C (nocturnal 8.0 ± 1.1 and 3.9 ± 0.2 vs 1.8 ± 0.4 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$; postprandial 2.4 ± 0.3 and 3.0 ± 0.3 vs 1.1 ± 0.1 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, all $P < 0.001$). Calculated as the hourly postprandial R_a :nocturnal R_a ratio, the relative postprandial abnormality in R_a was greater than the nocturnal abnormality in DM ($P < 0.05$), but not in DYS. Two hour postprandial (2HPP) FFA concentrations were greater in DM and DYS than in C (130 ± 24 and 115 ± 22 vs 49 ± 6 $\mu\text{mol/L}$, both $P < 0.01$). There was no correlation between fasting FFA concentrations and fasting TGs in any group. However, 2HPP FFA concentrations correlated strongly with fasting TGs in C ($R = 0.613$, $P < 0.05$) and in DYS ($R = 0.631$, $P < 0.005$), but not in DM ($R = 0.061$, $P = \text{NS}$) subjects. These results indicate that abnormalities in postprandial adipose tissue lipolysis make an inordinate contribution to around-the-clock FFA economy in insulin resistant states such as DM. They also suggest that postprandial FFA availability may be an important determinant of total hepatic FFA delivery for VLDL TG synthesis.

CONTRIBUTION OF DIETARY FAT TO PLASMA FREE FATTY ACIDS DURING MEAL ABSORPTION

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Plasma free fatty acids (FFA) are elevated in poorly controlled diabetes and are thought to mediate insulin resistance and endothelial dysfunction. Little attention has been given to the potential contribution of dietary fat to plasma FFA during hydrolysis of chylomicron triglyceride (TG) by lipoprotein lipase (LPL). Animal studies have shown substantial spillover of LPL-generated FFA into the plasma FFA pool, suggesting that LPL-mediated fat storage is an inefficient process. In the present study, healthy subjects ($n=7$) were given a mixed breakfast (45% carbohydrate, 35% fat, 20% protein) after an overnight fast. Oleate rate of appearance (R_a) was determined with a continuous infusion of [$1-^{13}\text{C}$] oleate before and for 8 h after the meal. Chylomicron TG metabolism was studied during peak postprandial (PP) fat absorption (4.5 to 5.5 h after the meal) using a continuous infusion of an HPLC-purified, sterilized lipid emulsion labeled with [oleyl- ^3H] triolein. ^{13}C oleate was used as a tracer to measure the R_a of ^3H oleate (an indicator of spillover of LPL-generated fatty acids into the circulation) during this interval. Arterialized blood samples were analyzed for ^{13}C oleate enrichment, ^3H oleate specific activity and ^3H chylomicron TG specific activity. Plasma TG concentration increased from 62 ± 14 at baseline to 114 ± 32 $\text{mg} \cdot \text{dL}^{-1}$ at 4.5-5.5 h ($P < 0.05$). Steady-state conditions were achieved in oleate concentration, ^{13}C enrichment and ^3H specific activity during the PP sampling interval. During the baseline and PP sampling periods, plasma FFA concentrations were 456 ± 72 and 395 ± 96 $\mu\text{mol} \cdot \text{L}^{-1}$, respectively ($P = \text{NS}$). At 4.5-5.5 h, chylomicron TG concentrations were 22 ± 6 $\text{mg} \cdot \text{dL}^{-1}$ and fractional spillover of LPL-generated ^3H oleate (R_a ^3H oleate \div ^3H triolein infusion rate) was 4.5 ± 0.4 %. These results indicate that LPL-mediated storage of dietary fat in healthy subjects is efficient, with very little spillover of fatty acids into the plasma FFA pool. Whether LPL-mediated fatty acid storage is less efficient in insulin resistant states such as obesity and type 2 diabetes will require further study.

FAMILIAL CORRELATION, HERITABILITY AND RECURRENT RISKS FOR METABOLIC SYNDROME AND LIPID PROFILES IN TAIWAN CHINESE

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INTRODUCTION: Genetic study on metabolic syndrome is a great challenge, due to its complex traits and the pleiotropy manifestation of atherosclerosis. Familial aggregation and recurrent risks can provide the insight of possible genetic mechanism.

MATERIALS & METHODS: The Chin-Shan Community Family study was based on adolescent probands and their relatives (1,356 subjects) who were recruited from one junior high school in the community. Structured questionnaires and biochemical measures were obtained in standard procedures. Definition of metabolic syndrome was followed using the criteria defined by the Third Adult Treatment Panel, with a modification of the criteria for adolescent and Asian population.

RESULTS: Grandmothers had the highest frequencies (70%) in metabolic syndrome and various atherosclerotic risks. Three factors were found and thus explained 68% of the overall variance. Estimated heritability was the highest in LDL and cholesterol factor (0.36 and 0.40), then blood pressure/obesity factor (0.27), and insulin resistance/dyslipidemia (0.27). Recurrent risk among siblings was 2.95 (95% confidence interval [CI], 1.39-6.26). The adjusted odds ratio [OR] of proband's metabolic syndrome status was 1.99 (95% CI, 1.08-3.66). The adjusted odds ratios for the three factors for predicting metabolic syndrome were all significant, with highest risk in blood pressure/obesity factor (OR, 1.27, CI, 1.22-1.33), then insulin resistance/dyslipidemia (OR, 1.29, CI, 1.16-1.23).

CONCLUSION: This study demonstrated clearly familial aggregation and recurrent risk of metabolic syndrome and components among the general ethnic Chinese population in Taiwan.

FENOFIBRATE (FENOFIBRIC ACID) EXPOSURE AND MAGNITUDE OF TRIGLYCERIDE (TG) RESPONSE FROM NHLBI SPONSORED GENETICS OF LIPID LOWERING DRUGS AND DIET NETWORK (GOLDN) STUDY

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Purpose: To determine the relationship between systemic exposure to fenofibric acid (FA) (active moiety of fenofibrate (FB)) and hyper- or hypo-responders to FB in terms of triglyceride response. **Design:** Subjects whose baseline TG exceed 150mg/dL, took FB 160mg once daily for 21 days. TG response was calculated by the change in TGs with treatment (post- minus pretreatment) expressed as a % of pretreatment TG level. FA serum concentrations at 0, 3.5 and 6 hours on day 21 were used to calculate a partial area under the serum concentration-time curve (AUC₀₋₆). **Results:** 159 subjects (86 males), median \pm SE age of 56.4 \pm 1.0 years were sorted by quintiles of TG response. Median \pm SE pretreatment TG, LDL-C, HDL-C, and TC for 32 hyper- vs 32 hypo-responders (from upper/lower quintile of TG response) were 248.0 \pm 33.1, 137.0 \pm 5.5, 42.5 \pm 2.0, 226.5 \pm 7.5mg/dL vs 193.5 \pm 11.7, 137.5 \pm 4.3, 46.0 \pm 1.8, 212.0 \pm 5.4mg/dL (NS between groups). Table: Median \pm SE ($\dagger p < 0.05$ by Wilcoxon test, Sex NS by χ^2)

Parameter	Hyper-responders	Hypo-responders
Age (n=males)	59.3 \pm 2.3 (16)	62.9 \pm 2.3 (24)
TG % change \dagger	-66.4 \pm 0.9	-19.9 \pm 2.9
LDL-C % Change	-16.4 \pm 5.8	-7.1 \pm 2.3
HDL-C % Change \dagger	13.7 \pm 2.4	6.4 \pm 2.1
TC Change % \dagger	-21.7 \pm 2.2	-8.1 \pm 1.5
FA AUC 0-6(mcg/mL*h) \dagger	84.3 \pm 4.8	60.3 \pm 3.6

Conclusions: We report an association between fenofibric acid AUC₀₋₆, and hyper- and hypo-responders to FB in terms of TG response. This suggests that FB absorption, distribution, metabolism and/or elimination are important determinants of the variation in TG response.

EFFECTS OF LIFESTYLE MODIFICATION ON ENDOTHELIAL DYSFUNCTION AND CVD RISK FACTORS IN THE METABOLIC SYNDROME AND TYPE 2 DIABETES MELLITUS

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Several prospective studies have shown that lifestyle modification (ILS) in the form of weight reduction and increased physical activity can decrease the rate of development of type 2 diabetes (DM2) in people with impaired glucose tolerance (IGT). It may also decrease the rate of development of the Metabolic Syndrome (Met Synd). However, long term effects of ILS to reduce cardiovascular events in people with the Met Synd of DM2 have not yet been determined.

In the Diabetes Prevention Program (DPP) 53% of the participants met the ATP III criteria for Met Synd at the baseline evaluation, whereas 47% did not. The effects of treatment with metformin or ILS on both the development and reversal of the Met Synd has now been reported. Both treatments were effective in preventing and reversing Met Synd in this high risk population, with ILS being more effective than metformin.

We have used high resolution ultrasound to measure the endothelium-dependent flow mediated dilation of the brachial artery (FMD) and the endothelium-independent response to sublingual nitroglycerine (TNG) in obese, insulin resistant subjects before and after ILS programs lasting six months to one year. These programs, which target a 7-10% loss of body weight and 150-175 minutes of moderate intensity exercise each week, have resulted in highly significant improvements in FMD without changing the response to TNG. There have also been improvements in selected serum markers of endothelial activation and inflammation.

These results suggest that ILS programs, either alone or in combination with medications, may reduce the development of the Met Synd in high risk individuals and may also reduce CVD risk in people who already have the Met Synd or DM2. Thus, increased physical exercise and weight reduction should be the cornerstone of therapy for these conditions.

EFFECTS OF PIOGLITAZONE IN FAMILIAL COMBINED HYPERLIPIDEMIA (FCH)

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Objective. Familial combined hyperlipidemia (FCH) is characterized by elevated levels of total cholesterol, triglycerides and apolipoprotein B and associated with insulin resistance. We hypothesized that pioglitazone treatment of FCH patients might improve insulin sensitivity, but may also have beneficial effects on serum lipid levels, body fat distribution, intramyocellular lipids and endothelial function.

Methods. In a double blind, randomized, cross-over study 16 weeks pioglitazone treatment (30 mg) was compared with placebo. Insulin sensitivity was measured using the hyperinsulinemic euglycemic clamp procedure, body fat distribution and intramyocellular lipids using MR-techniques and endothelial function using flow mediated vasodilatation.

Results. Pioglitazone improved insulin sensitivity (M-value 37.7 \pm 3.6 vs 33.0 \pm 3.3 mol \cdot min⁻¹ \cdot kg⁻¹ during placebo, $P < 0.05$) and LDL composition by increasing the K value (-0.11 \pm 0.06 vs -0.20 \pm 0.06 during placebo, $P < 0.05$). However, pioglitazone did not affect other serum lipid levels. Endothelial function, body fat distribution and intramyocellular lipids were also not affected. In addition, pioglitazone was associated with a decrease in liver enzymes (ALT, alkaline phosphatase).

Conclusions. Pioglitazone treatment of FCH patients without type 2 diabetes mellitus increases insulin sensitivity, decreases liver enzymes and has a beneficial effect on LDL composition but a neutral effect on total serum lipid levels. The change in insulin sensitivity might be too small to induce changes in endothelial function, body fat distribution and intramyocellular lipids.

ADIPOSE TISSUE: A NEW TARGET FOR CARDIOVASCULAR DISEASE AND METABOLIC DISEASES. ADIPONECTIN AND OBESITY-RELATED DISEASES

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Visceral fat may play an important role in the development of metabolic syndrome as recently visceral fat accumulation has been recognized to be an essential component of metabolic syndrome definition. We analyzed the gene expression profile of visceral and subcutaneous fat by large scale random sequence analysis. Unexpectedly, adipose tissue, especially visceral fat, expressed a variety of genes for secretory proteins and most of them were biologically active molecules which were named adipocytokines. We found that mRNA of plasminogen activator inhibitor type 1 and heparin binding EGF-like growth factor are highly expressed in visceral adipose tissues. From comprehensive analysis of adipose specific genes, we found several novel genes and among them apM-1 might be the most important one, which encode adiponectin, a unique and multifunctional collagen-like protein and plasma levels decrease in the subjects with visceral fat accumulation. Adiponectin has been shown to have anti-diabetic and anti-atherosclerotic properties. In addition, this protein has a potent anti-inflammatory function. Namely, adiponectin strongly inhibits NF- κ B signaling through cAMP-dependent pathway in endothelial cells, resulting in the suppression of TNF- α -induced expression of adhesion molecules. Adiponectin has been also shown to induce the production and the secretion of IL-10 of macrophages which may induce the increased secretion and production of tissue inhibitor of metalloproteinase (TIMP)-1 by autocrine mechanism and may prevent acute coronary syndrome by the prevention of plaque instability. In this lecture, I would like to show essential roles of adipocytokines including adiponectin and visfatin, a novel adipocytokine in the prevention and the development of cardiovascular disease and metabolic diseases.

FAT DOES TALK TO THE ENDOTHELIUM

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Adipose tissue is a newly defined endocrine organ producing adipokines that are secreted into the circulation and bind to receptors which activate signaling pathways in target tissues. Key target tissues of adipokines include skeletal muscle, heart and liver, in all of which insulin has major actions and vascular tissue including endothelial cells, smooth muscle cells and monocytes/macrophages. Some adipokines such as tumor necrosis factor- α (TNF- α), plasminogen activator inhibitor-1 (PAI-1), and interleukin-6 (IL-6) decrease insulin mediated glucose uptake and activate pro-inflammatory and pro-thrombotic pathways that promote vascular injury. Angiotensinogen a precursor of angiotensin-II and leptin are also products of adipose tissue; both promote hypertension, as well as inflammation and potential vascular injury. In contrast adiponectin enhances insulin-mediated glucose uptake, enhances the effect of insulin to decrease hepatic glucose production and inhibits inflammatory pathways. In subjects with increased visceral adiposity the pro-inflammatory adipokines are elevated in the circulation while there is a decrease in adiponectin. We identified the presence of coronary endothelium-dependent flow abnormalities in an insulin resistant Mexican American cohort without traditional risk factors of coronary artery disease such as diabetes, hypertension, smoking or hypercholesterolemia. Surprisingly, this coronary flow abnormality correlated with body mass index (BMI), but did not correlate with blood pressure, lipids, measures of insulin sensitivity, or apolipoproteins. However, there was an inverse correlation of coronary endothelial dependent flow with circulating PAI-1 levels and a direct correlation with adiponectin. The PPAR- γ ligand, rosiglitazone increased plasma adiponectin and improved coronary flow; the improvement in both were directly correlated. These data suggest that the fat does talk to the endothelium by alterations in adipokine production.

DIABETES REDUCTION ASSESSMENT WITH RAMIPRIL AND ROSIGLITAZONE MEDICATIONS: THE DREAM TRIAL

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There is little question that in the 21st century the epidemic of Type 2 diabetes mellitus will have a devastating impact on many diverse populations and health care systems throughout the world. Primary prevention of diabetes in patients who are at high risk for this condition clearly has to be part of a comprehensive strategy to deal with this epidemic.

The DREAM trial is a large international prospective randomized controlled study designed to determine:

1. Does ramipril prevent Type 2 diabetes in people with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG)?
2. Does rosiglitazone prevent Type 2 diabetes in people with IGT or IFG? Ramipril and rosiglitazone will be evaluated in a two by two factorial design. The study was started in July 2001, has recruited 5,269 patients in 21 countries and will have three to five years of follow up. The primary outcome is the development of diabetes or death. Secondary outcomes include conversion of IGT to normal glucose tolerance, reduction in fasting plasma glucose, two-hour plasma glucose, or hemoglobin A1c, reduction in an aggregate cardio-renal outcome, change in beta-cell function and change in insulin resistance.

This study has 90% power to detect a relative risk reduction of 20% at three years and 17% at four-years of follow up.

In order to randomize 5,269 participants, 24,872 individuals were screened. Those individuals found not to be eligible for the DREAM trial, will continue to be followed in a prospective epidemiologic study called epi-DREAM.

The prevention of Type 2 diabetes should be a high-priority public health endeavour. Large, prospective clinical trials like the DREAM trial will improve our understanding of the pathophysiology of this condition and provide important insights that may be clinically relevant.

ABDOMINAL OBESITY: AN IMPORTANT TARGET FOR THE OPTIMAL PREVENTION OF CVD

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The prevalence of type 2 diabetes is showing a spectacular progression worldwide, a phenomenon largely resulting from the epidemic proportions reached by obesity in various populations of the world. However, physicians have been puzzled by the heterogeneity of obesity as not every obese patient is characterized by chronic complications such as type 2 diabetes, hypertension and coronary heart disease. In this regard, body fat distribution, especially visceral adipose tissue accumulation, has been found to be a key correlate of a cluster of diabetogenic, atherogenic, prothrombotic and inflammatory metabolic abnormalities now often referred to as the metabolic syndrome. This dysmetabolic profile is predictive of a substantially increased risk of coronary heart disease even in the absence of hyperglycemia, elevated LDL-cholesterol or hypertension. For instance, some features of the metabolic syndrome (hyperinsulinemia, elevated apolipoprotein B, and small LDL particles; the so-called atherogenic metabolic triad) have been associated with more than a 20-fold increase in the risk of ischemic heart disease in middle-aged men of the Québec Cardiovascular Study. From a risk assessment standpoint, we have reported that the "hypertriglyceridemic waist" phenotype (waist circumference ≥ 90 cm combined with triglycerides ≥ 2.0 mmol/L) was associated with a high likelihood (80%) of finding this cluster of metabolic abnormalities resulting from abdominal obesity. It is therefore suggested that the hyperglycemic state of type 2 diabetic patients may only represent the tip of a huge dysmetabolic iceberg largely explained by the high prevalence of abdominal obesity in our population. As we have found that waist circumference is a useful index of abdominal visceral obesity and of related metabolic complications, it is proposed that waist circumference should be systematically measured in all patients. Finally, until waist circumference and visceral obesity are identified as additional relevant therapeutic targets, it is proposed that clinicians will not optimally manage cardiovascular disease risk in a large proportion of their patients.

THE IMPACT OF DIABETES PREVENTION ON CVD RISK

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Type 2 Diabetes is associated with an increased risk of cardiovascular disease and has been equated as a cardiovascular risk equivalent by NCEP ATP III. In addition to the independent effect of diabetes and hyperglycemia, the hypertension and diabetic dyslipidemia seen in association with metabolic syndrome and diabetes further contribute to the development of premature cardiovascular disease. To date, six randomized studies have demonstrated the impact of therapies to delay or prevent the development of diabetes in high risk populations. The effects of diabetes prevention on the development of CVD risk factors has been well described and emerging data suggests a direct impact on the development of atherosclerosis and cardiovascular events. This presentation will review the existing data on diabetes prevention and its impact on CVD risk factors and events. Computer modeling of diabetes prevention further contributes to our expectations of CVD prevention and cost effectiveness of diabetes prevention efforts.

THE EFFECT OF WEIGHT LOSS ON THE METABOLIC SYNDROME: BEHAVIORAL STUDIES

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There is no single, specific treatment for the metabolic syndrome (MS). The goal is to reduce the risk of type 2 diabetes and coronary artery disease by attempting to improve the risk factors found in a given patient. Control of obesity is the primary therapy for the syndrome. This can be done with a hypocaloric diet and increased physical activity. The diet should stress a non-atherogenic regimen. The goal is to decrease weight and waist circumference 7-10%. This can be achieved with a caloric deficit of 500-1000 kcal/day. The diet should stress reduced saturated fat (<7% of total calories), reduced trans fat, dietary cholesterol <200 mg/day, a total fat intake of 25-35% of calories and low sodium. Most dietary fat should be unsaturated and simple sugars should be limited. Physical activity should be increased to at least 30 minutes/day, but preferably to 60 min/day. This should be moderate intensity activity. Resistance training at least twice a week is also helpful. If weight loss, reduction of abdominal obesity, and greater fitness is achieved, each of the risk factors that are part of the syndrome will improve. Behavior modification is aimed at helping individuals identify the problems and barriers interfering with their ability to achieve lifestyle change, lose weight and maintain the loss over time. No program wishing to treat MS can ignore this approach, because weight loss is very difficult to achieve and even more difficult to maintain. Three longitudinal trials have used lifestyle modification to attempt to prevent type 2 diabetes in obese individuals with impaired glucose tolerance. These are the DaQing, Finnish Diabetes Prevention Study, and the Diabetes Prevention Program. All three have reported the value of diet and exercise in lowering weight and reducing the conversion rate to diabetes. They have also improved risk factors common to the metabolic syndrome. These studies will be reviewed.

PLASMA TRIGLYCERIDES AND ECG ISCHEMIC DEPRESSION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background: Possibility of electrocardiography in diagnostic of ischemic heart disease is lower at patients with diabetes mellitus than at patients without carbohydrates disorders. Possibly, it connects with alterations on energy maintenance of transmembrane stream of the electrolytes that participating in myocyte action potential forming. One of signs of the broken energy substrates metabolism in diabetic heart is increased accumulation of triglycerides in myocyte cytosol, connected with the increased availability of fatty acids in conditions with insulin insufficiency. At a diabetes the enlargement of blood triglyceride levels is also consequence of insulin insufficiency and increased lipolys in adipose tissue. Accumulation of triglycerides in a myocytes at persons without diabetes reflects the degree of myocardial ischemia. Thus these conditions promoting accumulation of triglycerides can strengthen a myocardium ischemia. In our work at patients with a diabetes, we have analyzed relationship between a level low density lipoprotein (LDL), which are the basic triglyceride rich particles of plasma and a degree of segment ST depression on the electrocardiogram at the maximal stress-test.

Materials and methods: 29 patients with DM 2 types (middle age 56,8±0,97 years) have been diagnosed coronary heart disease by results of the standard treadmill test and confirmed by the data of a stress - echocardiography. All patients had diagnostic decrease of a segment ST on electrocardiography and wall motion abnormalities obtained at stress - echocardiography.

Results: At the correlation analysis the tendency to statistically significant positive relation between degree of segment ST depression and plasma level LDL ($r=0.413$, $p=0,079$) has been revealed.

Conclusion: At a diabetes processes promoting the raised synthesis of triglycerides are increase patterns of a myocardial ischemia during exercise.

CAROTID INTIMA-MEDIA THICKNESS IN A GENERAL POPULATION: ASSOCIATION WITH AGE, SEX LIPIDS, HYPERTENSION, DIABETES, AND ATHEROSCLEROTIC DISEASE.

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Carotid intima-media thickness (IMT) has been associated with atherosclerotic vascular disease and may be present before overt manifestations of ischemic heart disease, peripheral vascular disease, and stroke.

Between October 2000 and October 2001, 2369 subjects presented for a "wellness" examination that included a history and physical examination, laboratory tests, and an ultrasound determination of the carotid-intima thickness.

Men, diabetics, older subjects, cigarette users, and those on antihypertensive medications were more likely to have higher lipid levels, lower HDL levels and higher blood pressures, higher fasting glucose and greater IMT values. White subjects tended to have higher blood pressures than Hispanic and Asian subjects and had increased IMT values compared to Hispanic and Asian subjects. Those with a history of prior atherosclerotic vascular disease (ASVD) had higher IMT values, lipid values, blood pressures, were older and predominantly male. There was an increase in the IMT associated with the increasing number of risk factors (BMI, triglycerides, HDL, LDL, systolic blood pressure). A breakpoint IMT value of 0.7mm predicted 93% of those subjects with previous ASVD and only 7% of these subjects had IMT values less than 0.7mm. In a subgroup under the age of 30, 8 subjects had IMT values of 7mm or more, possibly placing them at an accelerated rate for an event. The use of carotid IMT in a general medicine setting may help identify subjects at risk for ASVD and may help stratify risk when combined with traditional risk factors (lipids, hypertension, and biomarkers).

TRIGLYCERIDES, HDL PLASMA LEVELS AND ADIPOSE TISSUE PARAMETERS IN MILD ARTERIAL HYPERTENSION

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The aim was to explore the relationship of plasma triglyceride (TG) and high density lipoprotein (HDL) levels with adipose tissue parameters in mild arterial hypertension.

Materials and methods. Anthropometric and adipose tissue morphometric data were investigated in 213 men about 22-53 years of age. The body absolute and relative fat amounts were estimated by anthropometry. Subcutaneous abdominal adipocytes were explored by Rodbell. Plasma TG and HDL levels were estimated by standardized laboratory methods.

Results. In obesity TG and HDL levels, TG/HDL value (153,2±71,9 mg/dl, 37,5±8,8 mg/dl, 4,2±2,2) were significantly more ($p=0.000$), than in nonobese pts (108,6±60,3 mg/dl, 43,7±10,1 mg/dl, 2,8±1,9).

In nonobese pts ($n=105$) plasma TG level positive closely correlated with waist-to-hip ratio ($p=0.000$), that indicated on determinant role of abdominal adipose tissue as maximal lipolytic active in their forming.

In obese pts ($n=108$) plasma TG level did not significantly correlate with waist-to-hip ratio ($p>0.1$), that indicated adipose tissue with expressed lipolytic activity is find not only in abdominal region, but it is in peripheral fat depots. Absence of significant correlation between TG plasma level and total body fat ($p>0.1$) in obese pts possibly points out the higher lipolytic activity in peripheral adipose tissue that become localized in the most enlargement adipocytes.

Importance of cells of peripheral adipose tissue in forming of lipoprotein spectrum is indicated by significant correlations of HDL cholesterol with fat cell size ($r= -0.491$, $p=0.038$) and fat cell number ($r=0.497$, $p=0.036$) in patients with II degree of obesity ($n=23$).

HORMONAL PATTERNS OF DYSLIPIDAEMIAS IN NON-OBESE TYPE II DIABETIC PATIENTS WITH ATHEROSCLEROSIS

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The aim of this study was to evaluate the hormonal patterns of dyslipidaemias (DLP) in non-obese patients (pts) with Type II diabetes mellitus (DM) and atherosclerosis (ATH). Group 1 (gr1) was composed of 30 normal subjects (age=54.90±2.03; means±SEM). Group 2 (gr2) consisted of 20 non-obese type II DM pts with hypertriglyceridaemia (type IV hyperlipoproteinaemia (HLP)) and ATH (age=53.19±1.91). Group 3 (gr3) consisted of 23 non-obese type II DM pts with mixed hyperlipidaemia (type IIB HLP) and ATH (age=56.73±1.96). There have been determined in serum, in fasting state: total cholesterol (CH), HDL-CH, atherogenicity coefficient (AC), triglycerides (TG), lipolytic activity (LA), lipoprotein fractions. Following have been determined in plasma during OGTT: glucose, insulin, insulin/glucose index (IGI), glucagon, C-peptide, somatotropin (STH), somatostatin (STS), ACTH, cortisol (F), aldosterone (ALD), β -endorphin. Both gr2 and gr3 pts, compared to gr1, had higher body mass, CH, TG, AC, and lower HDL-CH, LA, insulin (at OGTT hr1), IGI, STH (hr2), basal ALD. Gr2 pts, compared to gr1, had lower STH (hr1,hr2). Gr3 pts, compared to gr1, had higher glucagon (hr2), STS (hr1,hr2), F (hr1,hr2), and lower C-peptide (hr1), STH (hr0,hr2). Gr3 pts, compared to gr2 pts, had higher CH (8.22±0.19 vs 5.98±0.21 mmol/l), HDL-CH (0.95±0.05 vs 0.68±0.04 mmol/l), STS (hr1; 28.25±3.01 vs 20.60±1.59 pmol/l), STH (hr1; 44.6±5.7 vs 22.4±2.9 pmol/l), F at all OGTT points (hr0; 478.7±48.0 vs 361.1±26.5, hr1; 492.8±54.5 vs 348.5±37.5, hr2; 425.4±50.4 vs 286.3±26.2 mmol/l). Altered hormonal patterns have been observed in non-obese Type II DM pts with ATH and DLP, including decreased STH and elevated cortisol.

HTS ASSAY DEVELOPMENT ON AN ACAT2 MUTANT WITH ENHANCED ENZYMATIC ACTIVITY

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Acyl-CoA:cholesterol Acyltransferase 2 (ACAT2) is a transmembrane enzyme which catalyzes the formation of cholesterol esters secreted as apoB-containing lipoprotein particles. ACAT2-mediated cholesterol esterification is considered as one of the major pharmaceutical targets for therapeutic intervention of hypercholesterolemia and atherosclerosis, but the complexity of the protein and the currently available assay format based on TLC have restricted so far the accessibility of this important target enzyme to the high-throughput drug discovery process. We present here the design of a homogeneous fluorescent assay suitable for the identification of specific ACAT2 inhibitors.

A bioinformatic analysis of the human ACAT2 sequence allowed us to design a mutant of ACAT2, named Enhanced Activity-ACAT2 (EA-ACAT2) predicted to display an increased transferase activity. Recombinant wild-type ACAT2 (wt-ACAT2) and EA-ACAT2 were expressed in insect cells and purified by membrane fractionation and chromatographic separation. The two versions displayed a similar production yield, but the quaternary structure of purified EA-ACAT2 was apparently different in comparison with wt-ACAT2. The activity of both proteins was proved by TLC separation of radiolabeled products. A homogeneous assay with a fluorescent readout was developed to follow cholesterol esterification in 384 MTP format. In both assay formats, EA-ACAT2 demonstrated a 4-fold higher activity compared to wt-ACAT2. As a consequence, EA-ACAT2 might be an obligate alternative to wt-ACAT2 for the configuration of an HTS-compatible assay. The kinetic parameters of EA-ACAT2 were calculated and the possibility to detect a specific inhibition was demonstrated by using a reference inhibitor.

In conclusion, we provide evidence for the first time that a biochemically HTS-compatible fluorescent assay in 384 MTP format can be designed to monitor the activity of recombinantly expressed and purified ACAT2.

HTS ASSAY DEVELOPMENT FOR CARDIOVASCULAR-RELEVANT LIPASES

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The molecular regulation of HDL metabolism is influenced by several extracellular lipases, and among them hepatic lipase (HL) and secretory phospholipase A2-IIa (sPLA2-IIa) play a key role: HL hydrolyzes HDL triglyceride and phospholipids, generating smaller lipid-depleted HDL particles, while sPLA2-IIa is an acute-phase protein that could contribute to the development and progression of atherosclerotic lesions during acute and chronic inflammatory states.

Standard lipase activity assays (e.g., HPLC, TLC) have precluded so far the application of parallel screening of large compound libraries in a high-throughput format, hindering the discovery of novel therapeutic drugs directed against HL and sPLA2-IIa. Our experimental approach is intended to fill this gap, by generating homogenous lipase assays based on fluorescent readouts compatible with the high-throughput screening (HTS) criteria.

We expressed the human HL and sPLA2-IIa genes in insect cells and we purified the recombinant enzymes in a catalytically active form by using different chromatographic strategies. For both lipases, the enzymatic assays were designed with fluorogenic surrogate phospholipids and triglycerides. The reactions were assembled through a homogeneous two-step procedure in 384 MTP format with a final reaction volume of 30 μ l and monitored following the kinetic increase of fluorescence intensity.

The reaction conditions were optimized to maximize the enzymatic activities in the miniaturized format and the kinetic constants for the fluorogenic substrates were determined. Specific inhibition in the HTS assay conditions was proved by using reference inhibitors.

The designed conditions are directly adaptable to 1536 MTP format and fully compatibility with an automated robotic procedure.

The successful approach adopted to set up the homogeneous fluorescence-based enzymatic assays for HL and sPLA2-IIa in 384 MTP format represents an important progress to convey these two therapeutically relevant lipases into the drug discovery process.

LIPIDIC METABOLICAL DISORDER IN OBESE CHILDREN

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Obesity is a nutrition disorder increasing in children and adult life, being associated with various disorder of lipid metabolism. The aim of our study is to point out the spectrum of lipoprotein abnormalities in obese children.

Material: We included in our study a group of 46 children with ages between 6-17 years, with sex distribution 28% females and 72% males, coming from the rural areas (36%) and from urban areas (64%).

Method: We analyzed the group according to the following data: personal data, clinical examination, educational level, socio-economical status, anthropometrical data, level of the lipoprotein profile (total cholesterol TC, LDL-c, HDL-c, apolipoprotein AI and B), correlated with the body mass index (BMI). We used as statistical analysis in this study the "t" test, and the Pearson correlation index "r".

Result: Our group was divided into a risk group (RG) of 46 patients, and a control group (CG) composed of 38 patients. We found a level of total cholesterol in the RG in girls 4,83±0,97 SD and in the CG 3,28±0,87SD (p=0,0036) in boys from RG we found 4,94±0,74 vs.3,46±0,64 in CG (p<0,001). We found hypertension in 12% of the girls and in 52% of the obese boys compared with the results of the CG (p< 0,0001). Between TC and BMI we found a direct positive correlation (r=0,65). LDL-c in RG was higher for boys (p=0,0960) as well as for girls (p=0,0305), compared with CG. Apo AI were significantly lower in RG (p=0,0009) compared with CG. HDL-c was lower for boys and girls but with statistical significance for obese boys (p=0,0228). Apo B levels were higher for RG in girls vs in CG (p=0,1374), and for boys 90,58±7,09 vs 79,3±11,3 respective (p=0,0010). We found obesity in 43,2% in patients low educational level, 21,7% in those with medium educational level and 17,3% in the high educational level group.

Conclusion: We found lipoprotein abnormalities including elevated total cholesterol, LDL-c and Apo B, and a decrease in Apo AI in obese children. We found hypertension only in obese patients and obesity was correlated with a low educational level

SERUM LIPOPROTEINS IN CHILDREN WITH FAMILIAL HISTORY OF MYOCARDIAL INFARCTION

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A positive family history of premature coronary heart disease (CHD) is recognized as an independent predictor for cardiovascular death in first degree relatives. It seems justified to identify subjects at high risk during young age. The aim of the study was to assess the value of various lipoproteins and apolipoproteins measurements concerning the risk for future ischemic heart disease in the progeny with parental/maternal history of premature CHD registered in the Cardiovascular Rehabilitation Clinic, compared with age-matched controls. **Material:** We included in our study a group of 30 teenagers with ages between 15-22 years, with sex distribution 14 females and 16 males, who come from parents with history of myocardial infarction (MI) before the age of 55 years, diagnosed by typical clinical symptoms, enzyme patterns and electrocardiographic alterations. The control group was composed by 48 teenagers, healthy, without CHD. The study group was divided in two subgroups <18 />18 years. **Method:** We analyzed the group according to the following data: personal data, clinical examination, lipoprotein profile (TC total cholesterol, triglyceride, LDL-c, HDL-c, apolipoprotein A1, B). Statistical analysis was performed with student 't' test and we considered p<0,05 significant. **Result:** Our group was divided into one risk group (RG) 30 teenagers, and one control group (CG) composed of 48 teenagers. Total cholesterol levels were high in 23% in RG <18 years with mean values of 192,2 mg/dl±47,8SD and in CG 157,6 mg/dl±26,3SD (p=0,0105), compared 36,8% at the subgroup >18 years in the RG 205,8±53,1mg/dl vs 170,6mg/dl±23,7SD in the CG (p=0,0036). Triglyceride were higher in both subgroups of the RG, but >18 years (p=0,0048) it was statistically significant. HDL-c was lower in both age group in RG but >18 years (p=0,0596) it was statistically significant. LDL-c was higher in RG <18 />18 years significantly (p=0,0085). Apo A1 showed differences in RG vs CG but without being significant. Apo B was higher in RG <18 years (p=0,0366). In our study the ratios TC/HDL-c showed significant differences between RG and CG (p<0,0001); >18 years LDL-c /Apo B was significantly different (p<0,0001) and HDL-c /Apo A1 (p=0,0005) as well. **Conclusion:** In both age subgroups we found significant differences in the RG but especially in TC and in TC/HDL-c ratio. At the group >18 years we found significant difference for LDL-c/ApoB and HDL-c/ApoA1. Apolipoproteins seems to be considerable as risk indicators for children with positive CHD

COMPARISON OF LIPID & LIPOPROTEIN ASSESSMENT METHODS RELATED TO THE METABOLIC SYNDROME

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The purpose of this investigation was to determine the influence of analytical method on reported concentrations of plasma triglycerides (TG) and HDL-C, and the impact of analytical method on clinical identification of the metabolic syndrome (MS). Due to its association with the metabolic syndrome, LDL phenotype classification (A, B) were also compared. **Methods:** Duplicate plasma samples from 113 adult participants of a cross-sectional CVD research study were sent to 2 laboratories specializing in blood lipid/lipoprotein analyses. The laboratories utilize different assessment techniques; LAB 1 utilized an enzymatic method (TG, HDL-C) plus GGE (particle size, subclasses), and LAB 2 utilized NMR spectroscopy. Concentrations of TG and HDL-C, and classification for ATP III MS lipid criteria were compared between labs. **Results:** Mean values (±SEM) were 97±5 vs 104±5 mg/dl for TG and 42±1 vs 46±1 mg/dl for HDL-C (both p<0.05) for LAB 1 and 2, respectively. Rank order values between laboratories were highly correlated (r=0.96, TG; r=0.91, HDL-C, both p<0.001). Eleven vs. 14 individuals met the TG criteria and 70 vs. 48 % met HDL-C MS criteria with LAB 1 and 2, respectively. LAB 2 characterized more individuals as LDL pattern B phenotype, as compared to lab 1 (30 vs. 14%, P<0.05). **Conclusions:** LAB 2 reported significantly higher mean values for TG and HDL-C, yet rank order results were highly correlated between labs, suggesting a consistent measurement difference. Lipid and lipoprotein analysis techniques may impact the proportion of individuals identified clinically as meeting the MS syndrome lipid and lipoprotein criteria as well as those with the LDL pattern B phenotype.

HYPOTRIGLYCERIDEMIA, INSULIN RESISTANCE AND LIVER DYSFUNCTION IN FAMILIAL HYPOBETALIPOPROTEINEMIA

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Familial hypobetalipoproteinemia (FHBL; OMIM 107730) is an autosomal co-dominant disorder of lipoprotein metabolism characterized by decreased plasma concentrations of apolipoprotein (apo) B-containing lipoproteins and resistance to atherosclerosis. However, the liver is an insulin-sensitive organ and impairment of VLDL secretion in FHBL may result in decreased plasma triglyceride concentrations, hepatic steatosis, and insulin resistance. We examined the effect of FHBL on lipids, apoproteins, insulin sensitivity and liver dysfunction. When compared with 48 unaffected family members, 39 FHBL subjects [apoB-6.9 (n=4), apoB-25.8 (n=3), apoB-40.3 (n=3), apoB-80.5 (n=4), R463W (n=16), and L343V (n=9)] showed decreased total cholesterol (2.5±0.1 vs. 4.6±0.2 mmol/L), LDL-cholesterol (0.9±0.1 vs. 2.9±0.1 mmol/L), triglyceride (0.5±0.1 vs. 1.0±0.1 mmol/L), apoB (0.28±0.02 vs. 0.89±0.04 g/L), and apoC-III (76±7 vs. 125±7 mg/L) concentrations (all P<0.001). Plasma free fatty acid concentrations were unchanged. Homeostasis model assessment (HOMA) score, an estimate of insulin sensitivity, was increased in FHBL subjects (2.3±0.4 vs. 1.1±0.1) as were serum markers of liver function, including alanine aminotransferase (59±8 vs. 26±3 U/L), γ-glutamyltransferase (50±9 vs. 21±2 U/L), and ferritin (191±29 vs. 104±13 μg/L) when compared with unaffected subjects (all P<0.005). We conclude that FHBL is associated with hypotriglyceridemia, insulin resistance and liver dysfunction secondary to decreased hepatic VLDL secretion.

PARTICULARITIES OF ADIPOSE TISSUE DISTRIBUTION IN PATIENTS WITH PRIMARY DIABETES MELLITUS TYPE 2 AND INSULIN RESISTANCE SYNDROME

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The aim of the work was to study the particularities of adipose tissue distribution in patients with primary non-insulin-dependent diabetes mellitus (NIDDM) using the data of computerized tomography of the abdominal cavity organs and subcutaneous cellular tissue.

The comparison of subcutaneous adipose tissue and visceral adipose tissue areas in men aged 40-60 years with abdominal obesity and NIDDM (Group 1) and healthy men of the same age (Group 2) was performed. The computerized tomography results are the following:

Parameter	Group 1 (n=20)	Group 2 (n=20)
1. Visceral adipose tissue area, cm ²	206.7±2.6*	150.9±11.1
2. Subcutaneous adipose tissue area, cm ²	236.5±18.0	233.9±19.0
3. Abdominal fat area, cm ²	443.0±29.9	384.9±24.4
4. Sagittal diameter, cm	13.1±2.5*	10.9±0.15

* - p<0.05

The patients of Group 1 showed a lower level of α-cholesterol and a higher level of triglycerides in blood and a significant increase in postprandial insulinaemia as compared to Group 2 patients. In addition, according to computerized tomography of abdominal region of Group 1 patients, they had significant accumulation of visceral fat around the portal.

ELEVATED TRIGLYCERIDE LEVELS, MARKERS / RISK FACTORS FOR CARDIOVASCULAR DISEASE

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High prevalence of T2DM and metabolic syndrome (MS) pose a high problem to increase cardiovascular disease in Asian Indians.

Study on 303 individuals of rural population compared with 90 individuals of urban for incidence of diabetes or increased markers of MS, the control group with MS of both urban and rural population showed significantly increased triglyceride (TG) levels (1.85 ± 0.58 vs 1.38 ± 0.48 , $p < 0.02$ – urban) & (1.51 ± 0.29 vs 0.98 ± 0.34 , $p < 0.001$ – rural) and low HDL cholesterol (HDL-C) (1.18 ± 0.15 vs 1.37 ± 0.25 , $p < 0.01$ – urban), (1.09 ± 0.26 , $p < 0.001$ – rural). Common trend of increased TG and decreased HDL-C was observed in T2DM, hypertensives and diabetes with hypertension with MS compared to controls without MS in both the urban and rural population. One important point to note is that in the control subjects without MS, TG level in the urban population was significantly high compared to rural population. (1.38 ± 0.48 vs 0.98 ± 0.34 , $p < 0.001$). Waist measurements are increased to 57% in urban population as compared to 6.49% of rural. Significant increase in TG levels in 31 % of urban population as 5.78 % in rural.

In comparison to sedentary lifestyle of urban Indians, rural population follows laborious intensive work habits in their daily life, demanded by agriculture, fishing, poultry and cow herding.

ELECTROPHYSIOLOGICAL PARAMETERS OF THE HEART IN PATIENTS WITH THE METABOLIC SYNDROME AND PAROXYSMAL ATRIAL FIBRILLATION

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Atrial fibrillation, in particular, its electrophysiological mechanisms, developing during the MS, have scarcely been studied.

The goal of this research was to study influence of the MS upon the electrophysiological parameters of the heart in patients with MS and AF.

Materials and methods. The research involved 58 cases with paroxysmal AF (35 females and 23 males average age $53,8 \pm 14,9$). The patients were divided into 2 groups. Group I involved 28 patients (19 females and 9 males average age $48,9 \pm 11,9$) suffering from AF with signs of the MS. Group II contained 30 patients (12 females and 18 males, average age $53,4 \pm 10,9$) with AF, but without the MS.

Results. In I group, the patients with paroxysmal AF demonstrated statistically significant ($p < 0,01$) refractoriness dispersion between the right atrium (RA) and left atrium (LA) (the RA effective refractory period (ERP) was about $177,8 \pm 25,3$ ms, and the LA ERP was $238,3 \pm 16,1$ ms). In II group patients with paroxysmal AF there wasn't refractoriness dispersion between the RA and LA. Echocardiography records in I group cases did not show visible increase in the front to back dimension of the LA ($3,7 \pm 1,4$ cm). In II group the LA front to back dimension was clearly increased ($4,2 \pm 1,9$ cm). Thus, refractoriness dispersion between the RA and LA in patients with MS and AF is one of the important factors for AF paroxysms development.

Conclusion. The above research results prove an immediate effect of metabolic syndrome upon the electrophysiology of the heart conductive system in patients with MS and paroxysmal AF.

METABOLIC SYNDROME PATIENTS WITH CABG AND ITS VASCULAR CONSEQUENCES

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Introduction: The prevalence of metabolic syndrome (defined by ATP III) is of 30 % in Europe and of 40% respectively in adult population above the age of 50 in USA. This syndrome (METS) sets the apparently healthy population-along with the patients with different entities of vascular disease-at high risk for a cardiovascular event. We investigated the prevalence of METS and the relation between its components and vascular damage in patients with history of coronary artery by-pass grafting (CABG).

Method: In our retrospective study on 258 patients with CABG (monovascular 11,3%; bivascular 20,5%; multivascular 68,2%) we assessed the R multiple coefficient (R square) for following parameters of METS: glycemia ≥ 110 mg/dl, high normal blood pressure 120-139/80-89mmHg, triglyceridemia ≥ 150 mg/dl, abdominal obesity (≥ 102 cm in men and ≥ 88 cm in women). We assessed the R multiple depending on: LDL ≥ 100 mg/dl, hypertension ($\geq 140/90$ mmHg), gender and age (men ≥ 55 years and women ≥ 65 years).

Results: METS had a prevalence of 52%. METS, defined through the four analyzed components, contributed to the severity of vascular damage in univascular men above 55 years (R square=0,33) and in hypertensive bivascular men (R square= 0,36). Multivascular patients with the four components of METS had a significant increase of triglycerides in relation with hypertension ($p=0,003$) with LDL ≥ 100 mg/dl ($p=0,0002$) and with gender and age-men above 55 years old ($p=0,003$).

Conclusions: In patients with CABG the presence of METS was in relation with severe vascular damage. By adding other risk factors to the four METS analyzed components, the severity of coronary artery disease (CAD) increased. In patients with CAD, the presence of both high triglyceride levels and other major risk factors may be considered as an indicator of high vascular risk.

SICK SINUS NODE SYNDROME IN PATIENTS WITH METABOLIC SYNDROME

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Sick sinus node syndrome (SSNS) is a rare finding in patients with metabolic syndrome (MS), therefore its electrophysiological have scarcely been studied. The goal of this research was to study electrophysiological peculiarities of SSNS in patients with MS.

Materials and methods: The research involved 35 cases with SSNS (21 females and 14 males average age $57,3 \pm 15,8$). The patients were divided into 2 groups. Group I involved 14 patients (9 females and 5 males average age $45,7 \pm 8,5$) suffering from SSNS with signs of the MS. Group II contained 21 patients (12 females and 9 males, average age $51,9 \pm 12,1$) with SSNS, but without the MS. The patient of both groups underwent invasive electrophysiological study. Permanent pacemakers were implanted in all the cases (in 9 (37,5%) cases rate-adapted SSIR type pacemakers, in 5 (20,8%) – DDDR type dual chamber pacemakers)

Results: In I group, the electrophysiological parameters (EP) of the sinus node (SN) function were significantly ($p < 0,001$) more suppressed than in group II. In particular in I group SN recovering time (SNRT) was $2135,7 \pm 115,5$ ms, SN recovering corrected time (SNRCT) – $831,3 \pm 91,7$ ms, sino-atrial conditioning time (SACT) – $487,1 \pm 73,8$ ms. In II group SNRT – $1930,9 \pm 98,3$ ms, SNRCT – $673,4 \pm 41,8$ ms, SACT – $401,1 \pm 35,7$ ms.

Conclusion: The research results prove that metabolic failures produce direct effect upon the SN function, causing suppression of its function as the SSNS develops, which requires implantation of pacemaker.

LIPOPROTEIN PROFILE, CAROTID WALL THICKNESS AND IMPAIRED ENDOTHELIUM-DEPENDENT VASODILATION IN SUBCLINICAL HYPOTHYROIDISM

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Fifty non-smoking women were investigated with age 55 ± 11 .

We measured lipoproteins, intima-media thickness and endothelium-dependent vasodilatation in 31 SHT patients in comparison with 24 euthyroid controls.

Total cholesterol (TC), triglyceride, high-density lipoprotein (HDLc) cholesterol, low-density lipoprotein cholesterol (LDLc) were measured. SHT patients showed significantly higher serum TC ($P < 0,01$), LDLc ($P < 0,01$) levels than controls, whereas no differences were noted for HDLc, TG concentrations.

To test the hypothesis that patients with SHT are characterized by endothelial dysfunction and to identify relationship between thyroid function and intima-media thickness in 31 SHT patients (serum cholesterol 211 ± 41 mg/dl) and 24 euthyroid subjects, subdivided into group I and group II (serum cholesterol, 167 ± 21 mg/dl and 215 ± 23 mg/dl, respectively) were studied IMT of carotid artery and endothelium-dependent vasodilatation of brachial artery.

The IMT of the carotid artery was measured noninvasively and accurately by ultrasound technique.

Examination of carotid wall thickness showed no statistically significant difference between euthyroid individuals (group I, group II) and participants with elevated serum TSH ($0,78 \pm 0,03$ mm; $0,91 \pm 0,05$ mm; and $0,82 \pm 0,03$ mm respectively. $p < 0,01$).

The TOSHIBA SSH-140A unit was employed to measure brachial artery (BA) diameter and blood flow velocity at rest and after occlusion. In SHT patients, vasodilation after occlusion was reduced, compared with group II ($5,1 \pm 0,05\%$; $7,8 \pm 0,03\%$; $p < 0,01$) and group I ($9,2 \pm 0,04\%$; $p < 0,01$).

THE POOLED PREVALENCE OF METABOLIC SYNDROME AMONG PATIENTS WITH SCHIZOPHRENIA

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Objective: Risk factors constituting the metabolic syndrome (obesity, dyslipidemia, hypertension, and hyperglycemia) are regarded as important prognostic indicators of new onset diabetes and cardiovascular disease (Sattar et al. *Circ* 2003;108:414-9). Due to lifestyle, patients with schizophrenia may be at uniquely elevated risk for metabolic syndrome and its clinical consequences.

Methods: A comprehensive search for all relevant studies presented/published since the definition of metabolic syndrome was released (September 2002) until September 2004. We computed the overall prevalence of metabolic syndrome, and average age of the combined population. This permitted the direct comparison of results to published estimates from the general population (Ford et al. *JAMA*. 2002;287:356-9), adjusted for age.

Results: A total of nine studies comprising 845 patients were identified. After adjusting for age, the prevalence of metabolic syndrome among the combined population was 39.4% (Mean age: 41.5 years). This estimate is double that of the general population (20%) after adjustment for age.

Conclusions: Patients with schizophrenia are at much greater risk for development of metabolic syndrome and its consequences. This is the first comprehensive estimate of metabolic syndrome prevalence in this population.

SIX MONTH INCIDENCE OF CONFIRMED DIABETES AMONG 200 TREATED SCHIZOPHRENIA PATIENTS ENROLLED IN A PROSPECTIVE COHORT IN BELGIUM

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Background: Diabetes risk among patients with schizophrenia has received growing attention. Although observational studies suggest an elevated rate of diabetes and glucose abnormalities in these patients, endpoint definition has often been inaccurate. We report the incidence of diabetes in a prospective clinic-based cohort using carefully measured laboratory metabolic parameters.

Methods: 200 schizophrenia (DSM-IV) patients free of glucose abnormalities were enrolled and followed for six months. Data collection included general clinical and demographic data and extensive metabolic screening (fasting glucose, insulin, and oral glucose tolerance tests). Data were collected at baseline, six weeks, three months and six months. Diabetes was defined as either a fasting glucose ≥ 126 mg/dl or OGGT glucose ≥ 200 mg/dl at 120 minutes.

Results: The mean age of the cohort was 37.2 years and 68% were male. Mean duration of schizophrenia was 14.1 years. The six month incidence of diabetes was 4% (95%CI: 1.3%-6.7%). Among a subset of 50 new patients, the six month incidence was 6% (95%CI: 0.1%-12.5%). This is much greater than the age adjusted incidence of diabetes in the general population (0.29%, National Center for Chronic Disease Prevention and Health Promotion – 1999)

Conclusions: The incidence of confirmed new onset diabetes among schizophrenia patients is much greater than that for the general population. Consideration of metabolic risks in schizophrenia patients is warranted and requires careful management.

INTERACTION OF ZN²⁺-IONS WITH NOT DIABETOGENIC CHELATORS PROTECT B-CELLS OF DESTRUCTION CAUSED BY XANTURENIC ACID, A DIABETOGENIC METABOLITE OF TRYPTOPHAN

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Among of 18 diabetogenic derivatives of 8-oxyquinolin (8OX) Xanturenic Acid (XA) only is synthesized in elderly human as abnormal metabolite of Tryptophan. XA as other 8OX caused diabetes due to ability to form in B-cells of toxic che-lat complexes with Zn²⁺-ions. **Goal of work:** to investigate protective effect of binding Zn²⁺-ions of B-cells by [A] – not diabetogenic chelator Na salt of Diethylthiocarbamic acid (NaDDA) in B-cells and [B] - by concurrent interaction of XA with Zn²⁺-ions dissolved in nutria medium. Isolated islets and B-cells were incubated in medium contained: [1] 289,4 - 416,2 mcg/ml of NaDDA 15 min. and then 48,8-53,6 mcg/ml of XA was added for 30 min incubation; followed incubation 6h in fresh nutria medium. [2] 0,05 ml of 1% solution of ZnSO₄ and 14,9-16,6 mcg/ml of XA was added 15 min later for 30 min incubation. [3]. Control: 39,3-52,7 mcg/ml of 8PTSQ, a diabetogenic derivative of 8OX. **Methods.** Staining of insulin (IN) in B-cells: immunohistochemical (IG), pseudoisocyanine (PS), immunofluorescent (IF) technics with measuring of density (D) of staining by IG, intensity of fluorescence (IF) of staining by PS. Staining of free Zn²⁺-ions: fluorescent method with 8para-(toluenesulphonylamino)quinolin (8PTSQ) and by Dihizon (DZ). Staining of IN+Histology: aldehydefuchsin technic (AF). Biochemical method: measuring of free XA in the nutria medium (XA-NM). Transmission electron microscopy of ultrastructures of B-cells (EM). **Results.** [3]. Disappearing of IN from cytoplasm of B-cells: IG-1,07+0,03; control-intact islets - 1,87+0,05; PS - 1,12+0,04; control - 2,24+0,07. Histology, AF: necrosis and destruction of 90-95% of B-cells in each islet; pycnosis of nuclei and degranulation of cytoplasm. Ultrastructures (EM): destruction of cell's matrix on 85-90% of cell's surface in 95-98% of B-cells; destruction of cover of B-granules, mitochondries and of endoplasmatic reticulum. [1]. Insulin content without changes: IG-1,79+0,04; control -1,84+0,05; PS-2,16+0,06; control -2,29+0,05. Histology, AF: necrobiosis of single B-cells (3-5% of total number of cells); other cells with partial slight decreasing of IN without histological changes. EM: destruction of cell's matrix, cover of B-granules and of endoplasmatic reticulum in 11-14% of total number B-cells; XA-NM: 2,3-2,9 mcg/ml; control (XA only)-12,2-12,8 mcg/ml. [2] Decreasing of IN in B-cells: IG-1,63+0,04; control-1,83+0,03; PS-1,98+0,06; control-2,21+0,03. Histology, AF: necrobiosis of B-cells (10-15% of total number) in 31-38% of islets, in other islets without visible changes. We conclude that interaction of Zn²⁺-ions in cytoplasm of B-cells with not diabetogenic chelator-protector result more effective protection B-cells of formation toxic complexes XA-Zn²⁺ comparatively with preventive binding of XA with Zn²⁺-ions contained in solution ZnSO₄ and inactivation of XA in solution.

ASSOCIATION OF THE METABOLIC SYNDROME AND PROFILE OF INFLAMMATORY CYTOKINES IN LADA DIABETES.

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Type I diabetes is a disease with onset usually in childhood and adolescence but up to a third of patients will be diagnosed in adulthood over 35 years of age. For these patients, the term "latent autoimmune diabetes in adults" (LADA) has been coined. LADA patients have a few immunological and genetic characteristics which are different from patients with classic Type I diabetes. LADA patients also had fewer markers of the metabolic syndrome. Our study investigated whether features of the metabolic syndrome influence the levels of inflammatory markers. 28 patients (age range: 35-65, BMI – 29.3±3.4) with LADA diabetes were investigated. HLA risk alleles were analyzed. Islet cell-specific autoantibodies: ICA, GAD and IAA and C-peptide levels were detected. CRP, IL-1, IL-2, IL-8 beside a full lipid profile and HbA_{1c} were measured. Hypertension (defined as RR>150/90 or treatment with antihypertensive drugs) and hyperlipidaemia (defined as fasting cholesterol >5.2 mmol/l, fasting triglycerides >1.7 mmol/l) were analyzed as markers of a metabolic syndrome. 15 healthy volunteers (age-range: 25-34, BMI – 25.2±1.7) served as controls. Our study has demonstrated increased levels of IL-1, IL-2 and IL-8, but CRP was not raised. Inflammatory cytokines were positively associated with an adverse lipid profile in LADA diabetes.

THE METABOLIC SYNDROME, INSULIN RESISTANCE, AND CARDIOVASCULAR RISK IN DIABETIC AND NONDIABETIC PATIENTS

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Background: The contribution of insulin resistance (IR) *per se* to the vascular risk inferred by the metabolic syndrome (MetS) is not known; conversely, it is uncertain whether IR infers vascular risk beyond the entity of the MetS. Further, the impact of the MetS on future vascular events among men with established coronary artery disease (CAD) has not been investigated yet, and data are scarce for patients with type 2 diabetes (T2DM). **Methods:** We therefore enrolled 750 consecutive patients undergoing coronary angiography for the evaluation of CAD and diagnosed the MetS according to current Adult Treatment Panel III criteria; IR was estimated by the homeostasis model assessment index.

Results: Over 2.3 years, both the MetS and IR predicted vascular events after controlling for non-MetS risk factors (hazard ratios [HR] 2.74 [95% CI 1.71-4.39]; p <0.001 and 1.51 [1.24-1.84]; p <0.001, respectively). After further adjustment for IR the MetS remained significantly predictive of vascular events (HR 2.69 [1.57-4.64]; p <0.001), and, conversely, IR remained significantly predictive of vascular events despite adjustment for the MetS (standardized HR 1.41 [1.14-1.75]; p = 0.002). In subgroup analyses the MetS significantly predicted vascular events among men with angiographically proven CAD (n = 350; HR = 2.18 [1.28-3.72]; p = 0.004) and among patients with T2DM (n = 164; HR 4.51 [1.29-15.75]; p = 0.018). **Conclusions:** We conclude that the MetS and IR are mutually independent predictors of vascular risk among angiographed coronary patients. Specifically, the MetS predicts vascular events among men with established CAD and among patients with T2DM.

CORRELATION OF HIGH-SENSITIVITY C-REACTIVE PROTEIN, FIBRINOGEN AND ULTRASONIC INDEX OF CAROTID ARTERIOSCLEROSIS IN PATIENTS WITH ACUTE CEREBRAL INFARCTION

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Objective: To explore the correlation of high-sensitivity C-reactive protein (Hs-CRP), fibrinogen (Fg) and ultrasound indexes of carotid arteriosclerosis in patients with acute cerebral infarction. **Methods:** A total of 103 subjects were divided into three groups: acute cerebral infarction group (ACI, n=33), old cerebral infarction group (OCI, n=34) and normal controls (n=36). The subjects' blood concentrations of Hs-CRP and Fg were measured while their carotid arteries were examined by color Doppler and B-Ultrasound. **Results:** The results showed that the carotid intimal-media thickness (IMT) was significantly increased in the two diseased groups as compared to the control group (P < 0.01), but no significant difference was found between ACI and OCI groups. The total plaque score (TPS) in OCI group was significantly higher than that in ACI and control groups (P<0.05). Moreover, the incidence rate of atherosclerosis plaque of carotid artery was increased in ACI group compared to OCI and control (P<0.05). The ACI group displayed a significant elevation in plasma Hs-CRP concentration relative to OCI and controls (P<0.01). The plasma concentration of Hs-CRP was stepwise elevating during 1-7 days in the ACI group. Plasma concentration of Fg showed no difference among the three groups (p >0.05). Multiple regression stepwise analysis revealed that the serum concentration of Hs-CRP was positively correlated with the plasma concentration of Fg and TPS, but was negatively correlated with blood resistant index. **Conclusion:** In the acute phase of cerebral infarction, a significant change in plasma Fg was not observed. Our results suggest that IMT and TPS is of clinical indexes of carotid atherosclerosis; the Hs-CRP is a risk of acute attack in cardiocerebrovascular events.

PREVALENCE OF METABOLIC SYNDROME AMONG PATIENTS WITH ANGIOGRAPHICALLY-DOCUMENTED CORONARY ARTERY DISEASE

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Background: Most Individuals who developed cardiovascular diseases have multiple risk factors. Clustering of these risk factor is called Metabolic Syndrome. There are limited data on the prevalence of Metabolic Syndrome among patients with angiographically- documented coronary artery disease (CAD).

Methods: This was a cross-sectional study of all adult patients who underwent coronary angiography at the UP-PGH Catheterization Laboratory for a year. Combined ATP III and Asia Pacific criteria of abdominal obesity were utilized.

Results: Seventy-eight (78) patients were found to have CAD. The prevalence of Metabolic Syndrome among patients with angiographically documented CAD was 40/78 (51.28%). Low HDL-C(90%), HPN(78%) and abdominal obesity (64%) were the most common clinical components of the metabolic syndrome in the study population. The following combinations were seen: 1) HPN, low HDL-C, abdominal obesity (15%) 2) elevated FBS, low HDL-C, abdominal obesity (15%) 3) HPN, elevated FBS, abdominal obesity, low HDL-C (15%). 73% of patients with 3-vessel CAD have metabolic syndrome. There was a significant association of Metabolic Syndrome and severe CAD (**OR 2.64 CI: 1.028, 6.758**).

Conclusions: The prevalence of metabolic syndrome among patients with angiographically documented CAD is 51%. Low HDL-C, HPN and abdominal obesity are the most common metabolic components in the study population. The risk of having 3-vessel CAD among patients with Metabolic Syndrome is 3x.

OBESITY AND THE METABOLIC SYNDROME ARE ASSOCIATED WITH PARTIAL ANDROGEN DEFICIENCY IN AGING MEN

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We evaluated the relationship between total serum testosterone (T), obesity, and the metabolic syndrome (MetS), using pooled baseline data in 858 middle-aged men (mean age: 52 years) from two lipid treatment studies. Inclusion criteria for the two lipid studies included low-density lipoprotein cholesterol ≥ 130 -160 mg/dL and triglycerides (TG) ≤ 350 mg/dL. Patients were defined as having the MetS if they met three or more of the following NCEP ATP III criteria: diagnosis of diabetes or fasting serum glucose ≥ 110 mg/dL or taking anti-diabetic medication; TG ≥ 150 mg/dL; high density lipoprotein cholesterol < 40 mg/dL; body mass index (BMI) ≥ 30 kg/m² (surrogate of waist circumference > 102 cm); and diagnosis of hypertension or blood pressure $\geq 130/85$ mmHg or taking anti-hypertension medication. For all patients, as well as the non-MetS and MetS cohorts, T decreased with increasing BMI (Pearson correlation coefficient: all patients, -0.37 [$p < 0.0001$]; non-MetS cohort, -0.27 [$p < 0.0001$]; MetS cohort, -0.41 [$p < 0.0001$]; $p < 0.05$ for correlations for non-MetS vs. MetS cohorts). T levels were generally lower in the MetS cohort compared to the non-MetS cohort, particularly for those men who were obese, i.e., BMI of 30 to < 40 kg/m² ($p = 0.007$). A multiple linear regression model was fitted with T being a dependent variable and BMI, MetS status, and age being independent variables. The model (with coefficient of determination of 0.11) and each of the independent variables were significant. A modification of this model, which included the status of each of the five MetS components as independent variables instead of overall baseline MetS status, was also fitted. Based on this modified model (with coefficient of determination of 0.10), TG ≥ 150 mg/dL, BMI ≥ 30 kg/m², and presence of diabetes were each found to be a significant predictor of lower T. This analysis suggests that obesity and the MetS are associated with partial androgen deficiency in aging men.

PLASMA PROCARBOXYPEPTIDASE U: A NOVEL COMPONENT OF THE METABOLIC SYNDROME

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Impairment of fibrinolytic function is a predisposing factor to coronary heart disease (CHD) and other atherothrombotic conditions. Carboxypeptidase U or activated thrombin activatable fibrinolysis inhibitor (TAFI) is a novel inhibitor of fibrinolysis, the clinical significance of which remains unknown. The relationships of plasma procarboxypeptidase U (proCPU) concentration to clinical and metabolic risk indicators for CHD were examined in 78 middle-aged healthy men with an apolipoprotein E3/E3 genotype.

Results: Among clinical risk indicators, body mass index BMI ($p < 0.001$) and waist to hip circumference ratio ($p < 0.05$) were significantly correlated with plasma proCPU concentration. Correlation analysis with metabolic risk indicators determined in the fasting state revealed a strong positive relation of proCPU with VLDL triglycerides ($p < 0.001$). Other metabolic markers that are perturbed in the insulin resistance syndrome, such as LDL particle size ($p < 0.05$), blood glucose ($p < 0.05$), HOMA estimate of insulin resistance ($p < 0.05$), plasma proinsulin ($p < 0.05$) and LDL triglyceride content ($p < 0.01$) were also significantly correlated with the plasma proCPU concentration. ProCPU also showed consistent positive correlations with plasma concentrations of large VLDL, large and small chylomicron remnants in the fasting state and postprandially after intake of a mixed meal.

The final multivariate model showed BMI to be the strongest determinant of proCPU concentration, accounting for 26% of the variation, whereas fasting plasma triglycerides proved to be the strongest metabolic correlate, contributing another 12% of the variation.

Conclusion: ProCPU can be considered as a component of the metabolic syndrome.

LIPID DISORDERS IN OBESE WOMEN

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Metabolic syndrome - Syndrome X is characterised by various lipid disorders. Women with android type of obesity are more frequent in group of person with metabolic syndrome, than women with gynoid type of obesity.

Aim: To correlate level of HDL cholesterol and triglycerides between women with android and women with gynoid type of obesity.

Methods: We analyzed lipid parameters: HDL cholesterol and triglycerides in group of obese women (N=31), BMI (34.71 \pm 1.14) aged 35.38 \pm 1.04) classified by anthropometrical parameter - waist/hip ratio (WHR) - into group A (android obesity: N=17; WHR > 0.85 ; body weight 90.35 \pm 2.97) and group B (gynoid obesity: N=14; WHR < 0.85 ; body weight 89.21 \pm 3.29). We excluded women with diabetes mellitus from examined group.

Results: Group A and group B did not differ significantly with respect to different WHR:HDL cholesterol (group A - 1.23 \pm 0.06 vs. group B - 1.18 \pm 0.08) and triglycerides (group A - 1.43 \pm 0.13 vs. group B - 1.85 \pm 0.40). In these two groups of obese women we did not find statistically significant correlation between lipid and anthropometrical parameters ($p < 0.05$).

Conclusions: Our results confirm significance of determination type of obesity in women, as risk factor for developing metabolic syndrome. Insufficient discrimination of index WHR and small number of evaluated women are possible reasons for our final results. Our findings must be assessed in greater number of obese women and with different anthropometrical parameters.

TWO NOVEL APOA5 MISSENSE MUTATIONS IN PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA

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Common variants of *APOA5* consistently show association with differences in plasma TG levels. Since there is emerging evidence that apoAV may affect TG lipolysis by stimulating LPL, we hypothesized that rare mutations of *APOA5* might explain the severe hypertriglyceridemia in patients where *LPL* mutations had been excluded. We sequenced the coding exons in *APOA5* of 28 Dutch patients with a diagnosis of Type I or Type V hyperlipoproteinemia. A female patient with Type V was heterozygous for a novel mutation G271C (c.811G>T). Pretreatment TG levels were 20 mmol/L. She was also homozygous for *APOA5**2 1131C, probably contributed to her hypertriglyceridemia. Her plasma apoAV level, measured by ELISA, was 5-10 μ g/ml, 20-fold higher than normal. Molecular weight estimation of the patient's apoAV was 3-fold higher than expected (110-120 kDa). This suggests that the Cys271 might form an intermolecular disulphide-bond with Cys227, resulting in apoAV homo-multimers, affecting the molecular weight and leading to higher plasma apoAV. Direct sequencing of *APOA5* coding exons in 93 Czech patients with plasma TG levels > 10 mmol/L revealed a male patient heterozygous for H321L (c.962 A>T). He was also heterozygous for S19W. Limited phenotype data was available and no relatives were available. 282 DNA samples from Czech individuals drawn from the general population were screened for this mutation, no carriers were found. Since limited information and family data exists for both missense mutation carriers, recombinant expression in *E. coli* of these variants followed by analysis of effects on LPL activation are underway, to clarify their functionality.

REDUCTION OF TIA BY ADDING NIACIN TO STATIN TREATED METABOLIC SYNDROME PATIENTS

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Background and Aims: Metabolic syndrome (MS) patients are at increased risk lifetime risk of macrovascular disease and its complications. Raising HDL is found more helpful than only achieving NCEP LDL-C goal in regression of atherosclerotic lesions in INDIAN subsets. **Materials and Methods:** We examined the efficacy of niacin to raise HDL-C in statin treated MS patients who are at irrespective of NCEP goal. Primary end points were all types of stroke and TIA and all cause mortality and morbidity. MS was defined as 3 or more of following NCEP ATP-3 criteria: 1) Triglyceride > 1.7 mmol/L; 2) HDL-C < 1.0 (MEN) 1.3 (WOMEN); 3) FPG – 6.1-6.9 mmol/L; 4) Blood pressure > 130/85mmHg; 5) BMI > 30/Kg/m²; 6) Waist circumference of >102(men) or 88(woman). **Results:** Of evaluated 108 patients with first episode of TIA on presentation 78(72%) had MS at baseline with 40 patients in statin + placebo arm VS 38 in statin +niacin arm. The efficacy of niacin was consistent with the total study cohort and patients with MS. Compared to statin+placebo NIACIN raised HDL-C and further reduced LDL-C, NON HDL-C, TG. Risk of recurrent TIA and subsequent stroke was reduced by 40% in niacin arm as against 28 % in placebo arm. Stabilisation and subsequent regression of atherosclerotic plaque was dramatic in niacin arm. **Conclusion:** Combination of niacin+statin was most effective in MS with macrovascular disease without any evidence of increased liver toxicity. The magnitude of HDL-C increase suggests that the site of action of these drugs is different and additive.

Lipid Variable	Statin+ Placebo	Statin+ Placebo	Statin+ Niacin	Statin+ Niacin
Mg/L	AT BASE	AT TAIL	AT BASE	AT TAIL
LDL-C	143	-(37-53)	147	-(44-62)
TG	262	-(88-108)	253	-(100-126)
Non-HDL-C	202	-(57-70)	196	-(63-79)
HDL-C	29	+(7-13)	27	+(20-26)

GOINO PROCEDURE AS POSSIBLE PROPHYLACTIC AGENT AGAINST CARDIAC DISORDERS ASSOCIATED WITH NON INSULIN DEPENDENT DIABETES MELLITUS

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Our previous study showed that GOINO Procedure (GP), combination mixture prescribed by specific ratio of Ganoderma Lucidum (GL), Coriolus Versicolor (CV) and Panax Ginseng (PG), lowered systolic blood pressure and LDL-cholesterol rather than any of three components as single therapy, and GP may be utilized as a possible prophylactic agent against cardiac disorders associated with Non Insulin Dependent Diabetes Mellitus.

Aim: This study was to specify protein, one of specific compositions of GP as well as a characteristic of extracted solution of GP compositions.

Method: 6g of GL, CV, PG were added to 1l hot water and boiled for 90 minutes. After boiling, filtered solution was centrifuged (2000/min for 10min.). Protein contained in the centrifuged GP solution was measured by SDS polyacrylamide gel slab electrophoresis. SDS-PAGE Molecular Weight Standards, High Rang, Prestained SDS-PAGE Standards, Low Range, Molecular Weight marker for SDS-PAGE, Ultra-low Range were used for measurements of molecular weight

Result: SDS resulted that protein contained in GP was found in 14KDa-66KDa migration. It also confirmed that extracted solution has an oxidation-reduction potential less than 330mV and an average of pH less than 6.5 pH.

Conclusion: GP extracted solution has a low value of oxidation-reduction potential and is a weak acid solution. GP contains low-molecular-weight protein as one of its various compositions and that may result to give impacts on blood pressure and cholesterol in NIDDM.

EFFECTS OF ROSUVASTATIN ON INSULIN SENSITIVITY IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA

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Objective: By influencing the mevanolate pathway, statins may have multiple effects besides lipid lowering. Studies that measured the effects of statins on insulin sensitivity directly by euglycemic-hyperinsulinemic clamp-technique have only been carried out in diabetic subjects. We evaluated the effect of rosuvastatin on serum lipids and insulin sensitivity in non-diabetic patients with familial combined hyperlipidemia (FCH), characterized by decreased insulin sensitivity.

Methods: In a double-blind randomized crossover study, 18 subjects with FCH (mean age 54 ± 7 year) were randomized to rosuvastatin 40 mg/day or placebo for 12 weeks. Blood samples were taken at baseline and after 4, 8 and 12 weeks of both treatment periods. Insulin sensitivity was determined with euglycemic-hyperinsulinemic clamp after 12 weeks of both treatment periods.

Results: Serum lipids and lipoproteins improved significantly (total cholesterol - 44%; LDL-c -50%; VLDL-c -56%, VLDL-TG -39%, triglycerides -28%) and both parameters of low grade inflammation (as measured by hsCRP, -16%) and oxidative stress (as measured by plasma-oxLDL, -55%) decreased markedly after rosuvastatin therapy as compared to placebo. However, the insulin sensitivity-index was unchanged (0.24 ± 0.07 vs. 0.25 ± 0.10 μmol.kg⁻¹.min⁻¹.(mU/l)⁻¹, placebo vs rosuvastatin *p*=0.71), and in concurrence, the HOMA index (2.12 ± 0.94 vs. 2.28 ± 0.91 mmol.mU.l⁻², placebo vs rosuvastatin *p*=0.45) was unaffected too.

Conclusion: Despite marked improvements in lipid- and lipoprotein values, low-grade inflammation and oxidative stress, a relatively high dose of rosuvastatin did not change insulin-sensitivity in subjects with FCH. According to our results, statin therapy can not prevent or postpone the onset of type 2 diabetes in insulin resistant subjects.

EFFECT OF CAPTOPRIL ON METABOLIC SYNDROME IN FRUCTOSE-INDUCED FATTY LIVER DISEASE

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Aim: To evaluate the effect of captopril on liver pathology, hepatic lipid level and hepatic oxidative stress occurring in rats given fructose-enriched diet (FED). **Methods:** 28 Sprague-Dawley rats were randomly divided into 2 groups: the animals in the control group were on chow diet and rats on fructose-enriched diet (FED). After three weeks FED rats were divided into 2 subgroups receiving either captopril, or a control group that received FED only. After two weeks systolic blood pressure was measured, plasma for various biochemical parameters was taken and the livers were examined. **Results:** FED rats developed hepatic macro- and microvesicular fat deposits with increase in hepatic triglycerides (+198%), hepatic cholesterol concentration (+89%), but decrease in hepatic phospholipids concentration (-36 %). As compared to the control group, the FED rats had increased hepatic MDA level, while α-Tocopherol concentration, Paraoxanase activity and the ratio between activity of Glut_{red} and Glut_{per} were reduced. Also observed in the FED rats were hyperinsulinemia (+87%), hypertriglyceridemia (+223%) and hypertension (+15 %). Captopril therapy reduced blood pressure (-24%), plasma triglycerides (-36%) and hepatic triglyceride levels (-51%). Captopril caused also an increase in hepatic phospholipids, α-Tocopherol concentration, Paraoxanase activity and the ratio between activity of Glut_{red} and Glut_{per}. **Conclusion:** Fructose diet is a risk factor for development of experimental fatty liver. Captopril may cause a decreased in the accumulation of hepatic triglycerides and an increase in the antioxidant status in liver of rats with fructose induced fatty liver.

DIET INDUCED CHANGES IN BLOOD LIPID AND INFLAMMATORY MARKERS

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An increasing number of individuals are placed on statin therapy in an effort to alter blood lipid and inflammatory profiles. Despite this, coronary artery disease (CAD) progresses in most pts, resulting in the need for stents and/or bypass operations. We reasoned that unless the underlying cause of dyslipidemia and inflammation was changed in our pts with CAD, long term outcomes would continue to be poor. We therefore enrolled 30 pts with Metabolic Syndrome and/or treated CAD in a unique diet and supplement program personally instructed by an MD, which included the requirements to eat one bag of dark salad greens/day, adding ½ cup of raw nuts daily, using only olive oil for cooking and dressings, and elimination or drastic curtailment of “white foods, combined with at least every other day flossing of teeth. Those pts who drank alcohol were encouraged to switch to daily red wine. 24/30 pts were taking statins prior to enrollment. All pts received 500mg Vit C 2X./day, B-100’s 1X/day, and extended release Niacin 1000mg/day. Baseline lipids and inflammatory markers were drawn at baseline and 3 months into therapy.

Results: Weight loss averaged 12+/-3 lbs. Total cholesterol decreased from 241+/-30 to 190+/-21: LDL decreased from 148+/-20 to 80+/-16: LDL III&IV percentage dropped from 33% to 21% (goal <20%); HDL increased from 40+/-10 to 68+/-8, while HDLIIb increased from 17% to 28% (goal >30%). Triglycerides decreased from 300+/-100 to 77+/-12. Hs-crp decreased from 2.0 to 0.08 while fibrinogen decreased from 460+/-30 to 277+/-22. Pt acceptance was high.

Conclusions: Dietary modifications including green leafy vegetables, raw nuts, and avoidance of white foods plus simple, inexpensive supplements drastically modify CAD risk factors, even among pts already taking statins.

THE EFFECTS OF PHASE II OF CARDIOVASCULAR REHABILITATION ON LIPIDS AND LIPOPROTEINS IN DIABETIC PATIENTS WITH AND WITHOUT SYMPTOMATIC CORONARY ARTERY DISEASE

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Objectives: We have compared the effects of phase 2 of cardiac rehabilitation (P2) on lipids and lipoproteins in diabetic patients with (group1) and without (group2) cardiovascular disease (CVD: coronary artery disease, stroke, peripheral occlusive vascular disease). **Method:** We have assessed the baseline serum lipids and lipoproteins and at approximately 90 days in 393 diabetic patients with (n=112patients) and without (n=281patients) CVD; We have defined the atherogenic dyslipidemia in diabetes mellitus as follows: TG \geq 150mg/dl, HDL < 40mg/dl men) or < 50mg/dl (women) and LDL \geq 100mg/dl; we have followed the performance of normolipidemic therapies in association with the other goals of P2. **Results:** a) On exit from P2 improvements in lipid profile were observed in diabetic patients with and without CVD who had had abnormal baseline values (based on the ESC-Guidelines) as follows: TC (-6mg/dl in gr.1, -10mg/dl in gr.2, p= NS), TG (-13mg/dl in gr.1, -9mg/dl in gr.2, p=NS), LDL (-4mg/dl in gr.1, -10mg/dl in gr.2, p=0.003), HDL (+2mg/dl in gr.1, +2.6mg/dl in gr.2, p=NS). There were not observed any statistic differences comparing the changes in participants with and without CVD, excepting LDL. b) The atherogenic dyslipidemia was more often in group 2, without significant differences (p > 0.5), both in men and in women at the end of P2. c) Including a fibrate in the P2, there was a significant reduction of TG levels (p=0.000001); statins led to lower LDL levels (p=0.006) and higher HDL levels (p=0.00001). **Conclusions:** Diabetic patients with or without CVD had a better lipid profile at the end of P2. Those without CVD had a better outcome regarding the lipid profile, especially the LDL level, than the patients with CVD. The diabetic patients with dyslipidemia but without CVD had better results of P2 than those with CVD. By including normolipidemic therapies in the P2, there is an optimization in outcomes of lipids and lipoproteins.

GENDER AND AGE RELATED DIFFERENCES IN TREATMENT AND CONTROL OF CARDIOVASCULAR RISK FACTORS IN HYPERTENSIVE PATIENTS WITH ANGINA

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Dyslipidemic, hypertensive patients (N=48,863) were stratified by gender, age, and angina (n=2,502) / non-angina (n=46,358) status. Confidence intervals (CI=95%) indicated significant differences in treatment and cardiovascular risk factor control between sub-groups. More men than women had LDL-cholesterol <100mg/dL (CI=angina 43.93-43.96 vs. 34.42-34.49 / non-angina 32.42-32.43 vs. 17.24-17.25) and \geq 100-<130mg/dL (CI=angina 32.11-32.14 vs. 35.10-35.17 / non-angina 53.85-53.86 vs. 32.43-32.44). More women than men had LDL-c \geq 130mg/dL (CI=angina 27.67-27.72 vs. 23.91-23.92 / non-angina 38.69-38.70 vs. 35.38-35.39). Women were less likely than men to receive: statins (CI=angina 69.95-69.99 vs. 82.11-82.13 / non-angina 59.80-59.80 vs. 63.72-63.72); any anti-lipidemic medication at all (CI=angina 25.93-25.97 vs. 13.48-13.48 / non-angina 36.73-36.73 vs. 30.73-30.73), or to have a cholesterol measurement in the past year (CI=angina 56.82-56.88 vs. 34.54-34.56 / non-angina 45.77-45.77 vs. 39.75-39.75). Primary care providers treat high-risk, hypertensive patients relatively aggressively. However, opportunities to forestall progression of cardiovascular disease were missed in hypertensive, dyslipidemic women - both with and without diagnosed angina - whose blood pressure and LDL-c were more likely to be uncontrolled.

CHROMIUM PICOLINATE AND BIOTIN COMBINATION REDUCES ATHEROGENIC INDEX OF PLASMA IN PATIENTS WITH TYPE 2 DIABETES

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The atherogenic index of plasma (AIP), defined as log (TG/HDL-C), has recently been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease (CVD). The nutrient combination of chromium picolinate and biotin (Diachrome[®]) has been previously shown to reduce insulin resistance and hyperglycemia in patients with type 2 diabetes (T2DM). The current study evaluated the effects of the combination of chromium picolinate (CP) and biotin on AIP in moderately obese subjects with T2DM using a 30-day, randomized, double blind, placebo controlled study design. Throughout the study, subjects continued to take stable doses of oral antidiabetic medication(s). At baseline, subjects had an HbA1c \geq 7% and at least a one-year history of T2DM. Thirty-six subjects with persistent impaired glucose control (2 hr glucose \geq 200 mg/dL) were randomized to receive actives (n=20, 600 mcg Cr as CP and 2 mg biotin/day) or placebo (n =16) for 30 days. Blood samples were collected for determination of plasma glucose and lipid profile. At the final visit, the active group had a significantly lower AIP compared to the placebo group (p<0.05). The AIP in the active group dropped from 1.15 to 1.06, whereas the AIP increased from 1.27 to 1.55 in the placebo group. A significant difference in triglycerides (p<0.02) and LDL-C/HDL-C (p<0.05) were also observed between the groups at the final visit. In the active group, the change in urinary chromium level was inversely correlated with the change in AIP (p<0.05). These results suggest that the nutrient combination of chromium picolinate and biotin may be a valuable nutritional adjuvant therapy to reduce AIP and correlated with CVD risk factors in people with T2DM.

STATIN AND ANTIHYPERTENSIVE PREPARATIONS COMBINE THERAPY FOR THE PATIENTS WITH METABOLIC SYNDROME

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The aim of the study was to evaluate the influence of statin and anti-hypertensive preparations combine therapy for the patients with metabolic syndrome.

Materials and methods. 36 patients (17 men, 19 women), age 38-56 yrs. (BMI=38.0±1.6 kg/m², waist circle=110.2±5.2 sm, triglyceride levels (TG)= 2.33±0.20 mmol/l) with metabolic syndrome (MS) were enrolled. Combine therapy was consist of mocsonidin 0.8 mg, indapamide 1.5 mg and atorvastatin 10 mg during 24 hours. The duration of therapy was 1.5 of month.

Results. As a result of this therapy systolic and diastolic blood pressure was significantly decreased (130.20±2.40 vs 158.10±5.30 and 80.30±1.30 vs 100.50±2.0, respectively, p<0.05). Significant decreasing of of HOMA-IR (3.51±0.13 vs 4.81±0.23), total cholesterol levels (5.21±0.11 vs 6.48±0.21 mmol/l, p<0.05), TG (1.47±0.16 vs 2.33±0.90 mmol/l, p<0.05), fibrinogen (2.78±0.12 vs 3.97±0.15 g/l) were registered. The HDL-C levels were increased significantly (1.20±0.08 vs 0.75±0.11 mmol/l, p<0.05). The decrease of microalbuminuria levels were registered at 30 % of patients.

Conclusions. This schema of combine therapy normalizes the levels of arterial blood pressure, lipid profile on the background of HOMA-IR and the anti-inflammatory factor (fibrinogen) that improves the remote prognosis of metabolic syndrome.

THE ACHIEVEMENT OF BLOOD PRESSURE GOAL IN METABOLIC SYNDROME PATIENTS: THE ACE INHIBITOR EFFECTS ON LIPID METABOLISM AND INSULIN RESISTANCE

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The aim of the present study was to investigate the effects of ACE inhibitor hypotensive therapy on the lipid spectrum and insulin resistance in the metabolic syndrome (MS) patients. 41 patients aged 40-59 of both sexes with MS (according to NCEP/ATP III criteria) were randomized into two groups: the study group (n=21) which received 10-20 mg of enalapril (Dr Reddy's) daily and the control group (n=20) which non-systemically received different hypotensive drugs. At baseline and after 12 week of the treatment the following parameters were measured: office BP level, waist circumference, fasting blood lipids, glucose and insulin levels before and after load of 75 g of glucose. The BP goals were achieved in 65% patients of the study group at mean enalapril dose 17 mg per day: SBP decreased on 13% and DBP decreased on 8%, while 65% of the control group patients showed a decrease of SBP and DBP on 4% and 2%, respectively. Body mass and waist circumference did not change in both groups of the patients. In contrast to non-controlled hypotensive therapy enalapril monotherapy led to decrease of insulin resistance as for the fasting and after load glucose to insulin ratios increased in the patients of the study group on 14 and 25%, respectively. While the control group patients showed the decrease of the fasting and after load glucose to insulin ratios on 12% and 5%, respectively. The majority of the MS patients had IIb type of hyperlipidemia. The enalapril monotherapy led to no changes in the total cholesterol (Ch) and LDL Ch concentrations, while triglycerides level decreased on 28% and HDL Ch concentration increased on 12%. The parameters of the lipid spectrum practically did not change in control group. Thus the enalapril monotherapy at dose of 17 mg per day in contrast to non-controlled hypotensive therapy led to the achievement of BP goal in the majority of the MS patients and was accompanied by unsulin resistance reduction, decrease of triglyceride concentration and increase of HDL Ch level.

EFFECT OF EZETIMIBE/SIMVASTATIN ON THE LIPID PROFILE OF HYPERCHOLESTEROLEMIC PATIENTS WITH METABOLIC SYNDROME

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The importance of lowering LDL-C in patients (pts) with metabolic syndrome (MetS) is widely recognized. The efficacy of ezetimibe/simvastatin (EZE/SIM) vs SIM alone in hypercholesterolemic (HC) pts with MetS was evaluated in an analysis of pooled data from 3 randomized, double-blind, placebo (Pbo)-controlled studies in which 3083 pts with LDL-C 145 - 250 mg/dL were randomized to: EZE/SIM 10/10, 10/20, 10/40 or 10/80 mg; SIM 10, 20, 40 or 80 mg; EZE 10 mg; or Pbo for 12 wks. The primary endpoint was % change in LDL-C for pooled EZE/SIM vs SIM. MetS pts had ≥3 of the following: BMI ≥30 kg/m²; TG ≥150 mg/dL; HDL-C <40/<50 mg/dL (men/women); HTN or blood pressure ≥130/85 mmHg; diabetes or fasting glucose ≥110 mg/dL. Of 2985 evaluable patients, 918 (31%) had MetS (366 on EZE/SIM, 384 on SIM, 87 on EZE, and 81 on pbo). The treatment effects in MetS and Non-MetS pts were similar and consistent with the entire cohort. EZE/SIM produced significantly greater improvements relative to SIM alone in the lipid/inflammatory profile of HC pts with MetS.

Parameter	Least Squares Mean % Change from Baseline				P-value EZE/SIM vs SIM
	MetS		Non-MetS		
	SIM	EZE/SIM	SIM	EZE/SIM	
LDL-C	-39.1	-52.0	-37.7	-52.6	P<0.001
Non-HDL-C	-35.5	-47.5	-34.4	-48.3	P<0.001
TG (median)	-22.7	-30.1	-16.7	-24.4	P<0.001
Apo B	-31.3	-41.2	-29.9	-41.8	P<0.001
HDL-C	8.5	10.5	6.5	7.3	P<0.050
CRP (median)	-16.7	-36.3	-14.3	-30.0	P<0.001

INFLUENCE OF MOXONIDINE ON INSULIN RESISTANCE AND LIPID LEVELS IN SUBJECTS WITH METABOLIC SYNDROME

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The correction of arterial hypertension significantly reduces the risk of cardiovascular diseases in subjects with diabetes mellitus and metabolic syndrome. However, the effects of different antihypertensive medications on insulin resistance are not fully investigated. Therefore, the aim of this study was to investigate the influence of imidazoline receptors agonist moxonidine on insulin resistance, lipid metabolism and plasma levels of interleukin-6 in patients with metabolic syndrome. Moxonidine was prescribed to 14 patients with metabolic syndrome and newly diagnosed arterial hypertension (age: 56.1±2.9 years, BMI: 30.81±1.06 kg/m², data are presented as mean±SEM) in dose 0.2-0.4 mg once daily for 9.2±0.6 weeks. Diabetes mellitus was diagnosed in 7 patients, impaired glucose tolerance – in 4 and 3 subjects had normal glucose metabolism. Glucose and insulin levels were measured at fasting and at 1 and 2 hours during glucose tolerance test. We found that at the end of the treatment period the insulin resistance was reduced which was reflected by significant decrease of plasma insulin levels at 1 and 2 hours after glucose administration, area under insulin curve – 6205.3±740.5 vs. 4464.6±477.1, p<0.05 and trend toward decrease of HOMA index – 3.77±0.6 vs. 2.85±0.47, p<0.1 before and after treatment, respectively. Moxonidine treatment resulted in reduction of plasma levels of interleukin-6, which is associated with insulin resistance – 2.0±0.7 and 0.46±0.15 pg/ml, p<0.05. However, there were no significant changes of plasma total cholesterol, LDL, VLDL, HDL and triglycerides levels after moxonidine treatment. We could conclude that the treatment with imidazoline receptors agonist moxonidine lead to improvement of insulin sensitivity in patients with metabolic syndrome, which could be implemented into the treatment of arterial hypertension in subjects with metabolic syndrome to correct the basic pathophysiological defect underlying this condition.

INFLUENCE OF THE METABOLIC SYNDROME ON MEAN AGE OF ONSET OF CORONARY HEART DISEASE IN MAURITIUS

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We studied the contribution of the metabolic syndrome (MS) and that of its components (as defined by the WHO (WHO/NCD/NCS/99.2)) in 467 Mauritian consecutive patients recruited in coronary care units, affected by CHD before the age of 60, who were compared to 299 healthy controls within similar gender and ethnic groups (population of North-Indian (NI) origin, South-Indian (SI) and mixed origin (Creole)).

The MS was found in 47% of 297 male NI patients, 49% of 74 SI patients, 48% of 45 Creole patients and 61% of 51 female NI patients. Amongst components of MS, abnormal glucose metabolism (AGM), HBP and low-HDL were risk factors (RF) for CHD in all groups, whereas obesity, central obesity and uricemia were RF for CHD in the NI group only; hyperinsulinemia and hypertriglyceridemia (HTG) were RF in NI and SI but not in the Creole group.

We also compared age of onset of CHD (AOC) between patients who were grouped according to their metabolic status (affected by MS or not; affected by its components or not). A trend was found towards a lower mean AOC in Creole CHD patients with the MS (45.1±6.5 v/s 48.0±7.6, p<0.18) compared to those without the MS, while an inverse trend was found in NI male patients (45.4±6.1 with MS v/s 44.4±6.7 without the MS, p<0.15). A higher mean AOC was found in female NI patients with the MS (49.2±5.2 v/s 45.2±6.8, p<0.02) and in SI male patients with the MS (46.8±6.0 v/s 42.0±7.9, p<0.004). Similarly higher mean AOC were found in male NI (p<0.005), male SI (p<0.003) and female NI (p<0.05) CHD patients with HBP. Higher mean AOC were also found in male NI (p<0.05) and male SI patients (p<0.02) with an AGM. With regard to HTG or low-HDL, mean AOC were similar in NI and SI CHD patients.

While the MS is a RF in half of NI and SI premature CHD patients, the other half without the MS seems to be affected by CHD at an earlier age.

PHYSICAL EXERCISE CAN BENEFIT METABOLIC DISTURBANCES IN OBESE DIABETICS

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Today dietary management, physical activity and drug therapy are partners in the battle to achieve and maintain low risk blood glucose, blood lipid and blood pressure levels in diabetics. The exercise training benefits insulin sensitivity and improves metabolic control, especially in obese type 2 diabetics. We have studied the effects of exercise training in a group of 20 obese women with type 2 diabetes (BMI>30), aged from 32 up to 55 years. They performed exercise sessions 3-4 times per week (brisk walking 30 min and/or swimming for 1 h). Total caloric intake was 1500-1800 cal/day for all subjects. Body mass index (BMI), waist/hip ration (WHR), HbA1c, serum levels of insulin, C-peptide and lipids status (cholesterol, triglycerides, LDL and HDL cholesterol) at the beginning of the study and after 6 months follow-up period were determined. During this study, obese diabetics reduced BMI (35.88±5.06 vs 32.22±4.45, p<0.05) and WHR (0.84±0.04 vs 0.80±0.05, p<0.01). The quality of metabolic control of diabetes, assessed by HbA1c determination, improved (8.82±3.05 vs 8.12±1.92, p<0.05). The results obtained showed the significant decrease of insulin (50.84±21.48 vs 36.61±17.03, p<0.05) and C-peptide (2.09±0.73 vs 1.64±0.47, p<0.05), cholesterol (8.25±3.66 vs 6.38±2.35, p<0.01), triglycerides (4.73±2.55 vs 2.47±2.30, p<0.01), LDL (4.08±1.27 vs 3.17±1.16, p<0.01) and the increase of HDL (0.90±0.30 vs 1.21±0.28, t=10, p<0.01) levels in the sera of obese diabetics. Decreased (90% vs 65%) rate of hyperlipoproteinaemia was found. The physical exercise can reduce the levels of total and abdominal fats as well as the insulin resistance with its compensatory hyperinsulinaemia. These results suggest that the administration of exercise training program leads to reduction of body weight and rate of abdominal fat, which can provide a gradual normalization of serum glucose and lipid levels in obese diabetics.

MEDITERRANEAN DIET ENRICHED IN EITHER NUTS OR OLIVE OIL REDUCES PLASMA AND VLDL CHOLESTEROL AND TRIGLYCERIDES: THE PREDIMED STUDY.

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Mediterranean diet (MedDiet), which following the Seven Countries Study, has been proposed as a healthy dietary standard its association with a low rate of cardiovascular mortality. Since the Seven Countries Study revealed that olive oil intake was a key factor in this diet, consumption of this oil has been emphasized. Recent studies have also associated nut consumption, which are typical of the MedDiet, with low cardiovascular mortality rate. Olive oil (OO) and a mixture of nuts (NUT) (walnuts, hazelnuts and almonds) were administered within a MedDiet to two groups of 16 volunteers with a high risk of cardiovascular mortality for 12 weeks. The effect of this diet on plasma and VLDL lipid composition was compared with a control group that followed the AHA guidelines. MedDiet+NUT accounted for a reduction in triglycerides (TG) and total and LDL-cholesterol concentrations in plasma. MedDiet+OO affected mainly VLDL concentration as VLDL-cholesterol and TG as well as total serum TG were reduced. In addition OO reduced the TG/apolipoprotein B ratio. OO did not modify total and LDL-cholesterol concentrations but increase that of HDL-cholesterol. These changes were concomitant to increases in the content of oleic acid (18:1 n-9) after NUT and α -linolenic acid (18:3 n-3) after NUT and OO. In conclusion, the present results support the protective effect of the MedDiet but point out that NUT and OO reduce risk factors by different mechanisms.

EFFECT OF ORLISTAT ON WEIGHT AND OTHER CARDIOVASCULAR RISK PROFILE IN OBESE PATIENTS: A META-ANALYSIS

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Background: Obesity induces metabolic abnormalities that contribute to increased risk in cardiovascular disease. Weight loss in obese patients improved other metabolic profiles. Orlistat, blocks intestinal uptake of fats and promotes weight loss.

Objectives: To determine a.) the effectiveness of orlistat on weight reduction among obese patients, and b.) the effect of orlistat on lipid profile, FBS, HbA1c, and BP levels among obese patients.

Method: Meta-analysis of 21 randomized-controlled trials on the effects of orlistat vs placebo on weight reduction and other metabolic profiles among obese patients after 1 year of treatment.

Results: Obese individuals on orlistat 120 mg/tab TID for a year compared to placebo had a mean reduction of 2.42 kg (95% CI: 1.87,2.98) p<0.00001, and favorable changes in metabolic profile as follows: FBS -0.65 mmol/L (95%CI -0.84,-0.45) p<0.00001, HgbA1c -0.31% (95%CI -0.40,-0.22) p<0.00001, Total cholesterol -0.34 mmol/L (95%CI -0.41,-0.27) p<0.00001, Triglyceride -0.09 mmol/L (95%CI -0.20,0.02) p=0.12, HDL -0.02 mmol/L (95%CI -0.04,-0.01) p=0.007, LDL -0.25 mmol/L (95%CI -0.32,-0.19) p<0.00001. Orlistat had favorable SBP changes, -1.73 mm Hg (95%CI -3.27,-0.18) p=0.03 and DBP changes -1.22 mm Hg (95% CI -2.35,-0.10) p=0.03. Regarding GI side effects, Orlistat has a relative risk of 2.25 (95%CI 1.71,2.97) p<0.00001.

Conclusions: In addition to weight reduction, orlistat has a favorable effect in the improvement of cardiovascular risk profile among obese individuals. Orlistat is well tolerated by majority of the patients.

ROLE OF LIFESTYLE MODIFICATIONS IN HYPERTENSIVE AND OBESE PATIENTS

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Introduction: hypertension plays an important role in cardiovascular disease. In the presence of hypertriglyceridemia and obesity, just a small increase in the level of blood pressure is necessary for cardiovascular disease to develop.

Objectives: the aim of the study was to evaluate the effect of a low caloric diet and regular aerobic exercise on systolic and diastolic blood pressure, lipid profile, waist-to-hip circumference (W/H) ratio and weight reduction.

Material and Methods: forty two overweight/obese subjects (BMI>27 Kg/m²), aged 38±12 years were studied. Anthropometrics parameters were measured before prescription of a low caloric diet adjusted to age, gender, physical activity and professional lifestyle. Implementation of aerobic exercise (40 minutes, 3 times for week) was performed as well. The average follow-up was 12±3 months. Blood pressure (BP) and heart rate (HR) parameters were calculated non-invasively during 10 minutes by Finapres® arterial wave. Blood was collected after 12 hours of fasting.

Results: first evaluation vs Last evaluation: Weight (kg) 81.3± 13.7 vs 73.5± 12.8 (p<0.05); BMI (kg/m²) 31.5± 4.8 vs 28.7± 4.6 (p<0.01); W/H 0.833±0.11 vs 0.781±0.09 (p<0.05); % Body fat 39.6± 8.5 vs 36.4± 8.1; SBP (mmHg) 142.8± 36.0 vs 123.8± 27.7 (p<0.05); DBP (mmHg) 80.9± 26.1 vs 66.4± 16.7 (p<0.01); HR (bpm) 74.6± 9.5 vs 68.7± 9.5; Glucose (mg/L) 102.0± 40.3 vs 87.1± 8.9 (p<0.05); Triglycerides (mg/L) 103.9± 53.6 vs 93.0± 32.2; T.Cholesterol (mg/L) 208.2±37.6 vs 193.0± 47.3; HDL(mg/L) 55.2± 17.3 vs 58.3± 18.6; LDL(mg/L) 127.8±34.3 vs 123.0±32.2.

Conclusions: low caloric diet and regular physical exercise induced significant improvement in blood pressure, body mass index, waist-to-hip-circumference, glucose, without any change on drug prescription.

PREVALENCE OF THE METABOLIC SYNDROME IN YOUNG ADULT FILIPINOS AGED 18 – 25 YEARS OLD: A PRELIMINARY REPORT

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Background: Metabolic syndrome (MetS) as defined by the WHO and NCEP-ATP III-IAS criteria is a clinical condition where there is a clustering of risk factors considered to correlate very well with future development of diabetes and cardiovascular disease. However there is paucity of available data about the prevalence of the metabolic syndrome in the younger population specifically 18-25 year olds in the Philippines.

Objective: To estimate the prevalence of the metabolic syndrome in young Filipino adults aged 18 – 25 years old.

Design, Setting, and Participants: Analysis of data on randomized students at 3 universities was done. 196 Filipino men and women aged 18-25 years completed the survey.

Main Outcome Measures: Prevalence of the metabolic syndrome as defined by WHO and NCEP-ATP III-IAS Harmonized guideline (≥3 of the following abnormalities): waist circumference greater than 90 cm in men and 80 cm in women; serum triglycerides level of at least 150 mg/dL (1.69 mmol/L); high-density lipoprotein cholesterol level of less than 40 mg/dL (1.04 mmol/L) in men and 50 mg/dL (1.29 mmol/L) in women; blood pressure of at least 130/85 mm Hg; or serum glucose level of at least 110 mg/dL (6.1 mmol/L), urine albumin excretion rate ≥ 20mcg/min, and insulin resistance measured as Homeostasis model assessment of insulin resistance

Results: The prevalence of the metabolic syndrome was 5.1% and 0.51% based on the WHO and NCEP-ATP III-IAS criteria, respectively.

Conclusions: These results showed a low prevalence rate of the Metabolic Syndrome using both the NCEP-ATP III-IAS and WHO criteria among Filipino young adults aged 18-25 years old.

EFFECT OF ATORVASTATIN ON LIPID PROFILE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND METABOLIC SYNDROME

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Background: It is well known that resistant dyslipidemia, connected with metabolic syndrome and type 2 diabetes mellitus is associated to very high cardiovascular risk in this population. **Aim:** The aim of the study is to evaluate and compare the effect of atorvastatin 20 mg on the lipid profile in patients with type 2 diabetes mellitus and metabolic syndrome. **Methods:** The study population was divided into two groups: patients with type 2 diabetes mellitus (28 patients) and patients with metabolic syndrome (12 patients). Atorvastatin 20 mg tablets were administered for the period of one year. Complete lipid profile – total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were evaluated. The lipid profile was re-evaluated after 6, 16 weeks and the year. The patients in both groups were advised behavioral modification and given atorvastatin. **Results:** after 6 weeks of treatment, atorvastatin significantly altered plasma lipid profile both in diabetics and metabolic syndrome groups and this effect persisted over the observed period. LDL-C has been decreased in diabetic mellitus and metabolic syndrome groups by 37,2% and 44,4% respectively, while HDL-C has been increased in both groups by 17% and 20% and plasma TG has been decreased by 35% and 40% respectively. **Conclusion:** In our study pharmacological therapy with Atorvastatin, as well as, life style modification is an efficacious treatment of atherogenic dyslipidemia in patients with type 2 diabetes mellitus and metabolic syndrome.

PREVALENCE OF METABOLIC SYNDROME IN A SAMPLE OF HYPERTENSIVE PATIENTS TREATED IN PRIMARY CARE

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Background and Aims: To establish the prevalence of metabolic syndrome (MS) in a sample of patients treated in primary care and possible relations to other factors. **Materials and methods:** Multicenter, descriptive, transversal, and open study in hypertensive patients with or without medical treatment, recruited by consecutive sampling. Metabolic syndrome (MS) was defined according to ATP III criteria. The T-Student distribution and ANOVA were used in the data analysis for paired groups, and the chi-square was used to analyse the relationship between qualitative variables. The values of quantitative variables were showed as mean±SD, and the values of qualitative variables as percentages, considering a margin of confidence of 95%. **Results:** 411 patients were recruited, 51.4% were women and 48.6% men, the mean age was 63.53±10.29 years for women and 61.94±10.85 for men. MS was diagnosed (at least three criteria fulfilled) in 54.4% of cases, of which 3 criteria in 28.2%, 4 criteria in 17%, and 5 criteria in 9.2% of cases. Correlations of MS with other variables were significant with the presence of diabetes (chi-square=43.271, 1g., p=0.000), as well as dyslipidemia (chi-square=42.573, 1 DF, p=0.000). Regarding the quantitative variables, there were significant differences between the two groups in the following cases: Diastolic BP (t=2.220, 359.81 DF, p=0.027), Glucose (t=9.519, 385.53 DF, p=0.000), creatinine (t=2.327, 298.62 DF, p=0.021) and uric acid (t=3.667, 379.00 DF, p=0.000). Finally, a significant logistic regression model was obtained (chi-square=91.218, 5 DF, p=0.000) with a profitability of 70.7 %, with MS as dependent variable in which a significant, led to OR for sex (OR=1.872), for diabetes (OR=5.727), for dyslipidemia (OR=2.814), for total cholesterol (OR=1.008) and uric acid (OR=1.247). **Conclusions:** The prevalence of MS in the sample of hypertensive patients was very high, and their correlation with other risk factors very significant. Thus, the cardiovascular risk of these patients is very high.

DIFFERENT LISTS OF RECOMMENDED INDO-MEDITERRANEAN FOODS TO TREAT DIFFERENT FEATURES OF METABOLIC SYNDROME (MetS)

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The “Medical Service Coronary Heart Disease Prevention Programme” is a programme that the Medical Service of FAO, in collaboration with CNR, offers to the staff of FAO, IFAD and WFP based in Rome (Italy), for males (M) ≥ 45 years of age and females (F) ≥ 50 years of age (< 50 if in premature menopause). The programme consists of collection of data via a questionnaire and screening for major risk factors; calculation of individual CHD risk profile over the next 10 years using a computerized programme based on data from the Framingham Heart Study; providing staff members with results of the tests including a comment and advice on a healthy life style; identification of subjects with a high risk profile (risk level $> 20\%$ in 10 years) or with multiple risk factors or with MetS and inclusion in a programme of a healthier life style and, where required, pharmacological treatment. 480 staff members (272 M and 208 F) took part in the study. If the 35% of M and the 46% of F ($p=0.01$) did not show any risk factors, the prevalence of the MetS was 19% in M versus 10% in F ($p=0.04$). The risk level in 10 years was 6.7% in subjects without MetS versus 12.7% in subjects with MetS ($p<0.001$). Dietary intervention has been based on promoting a healthier diet. To achieve this, different lists of recommended foods to treat hypercholesterolemia, hypertriglyceridemia-overweight, obesity, diabetes, hyperuricemia and hypertension, all with a common approach based on the Indo-mediterranean diet, have been developed in collaboration with the FAO Food and Nutrition Division and distributed to staff. The philosophy of the list of recommended foods is founded on the observation that the healthy effects from the different components of the Indo-Mediterranean diet produce their benefit only when combined into a whole dietary approach.

THE MULTIDOMAIN ORGANIZATION AND THE STABILITY OF HUMAN APOLIPOPROTEIN E2, E3 AND E4 IN SOLUTION

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The stabilities toward thermal and chemical denaturation of three recombinant isoforms of human apolipoprotein E (r-apoE2, r-apoE3, r-apoE4), human plasma apoE3, the recombinant amino-terminal domain (NT) and the carboxyl-terminal (CT) domain of plasma apoE3 in solution were studied. By far ultraviolet circular dichroism (UV CD), thermal unfolding was irreversible for the intact apoE isoforms and consisted of a single transition. The r-apoE3 was found to be less stable as compared to the plasma protein and the stability of recombinant isoforms was r-apoE4 $<$ r-apoE3 $<$ r-apoE2. The thermal denaturation of the isolated NT- and CT-domains of apoE3 was largely reversible and included two transitions. By near UV CD, the thermal unfolding was biphasic. When compared, thermal unfolding of the secondary and tertiary structures appeared to occur concurrently in r-apoE2 whereas heating affected the tertiary structure, initially, in r-apoE3 and r-apoE4. Denaturation with guanidine hydrochloride (GuHCl) did not follow a two-state transition. A three-state treatment of the denaturation curves revealed the order of stability as r-apoE4 $<$ r-apoE3 $<$ r-apoE2 for the whole proteins as well as that for the NT-domains, as established by fluorescence and far UV CD spectroscopy, whereas the CT-domains had roughly similar stabilities. The results show that, although all three isoforms possess a similar domain structure, there are isoform-specific differences in the stability and in the state of association and that the unfolding both of the NT- and CT-domains may be more complex than a simple two-state transition.

A 12-WEEK VERY-LOW CARBOHYDRATE DIET (VLCD) SIGNIFICANTLY REDUCES CASES OF THE METABOLIC SYNDROME IN MEN

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VLCD have been shown to improve cardiovascular risk by reducing bodyweight and triglycerides (TG) and by increasing HDL-C. A study was conducted to determine if the benefits of the diet could be enhanced by adding a soluble fiber (SF) supplement. In the double-blind, parallel arm study, 29 overweight or obese men (BMI 25-38) aged 20-68, were matched according to BMI and age and were randomly assigned to either SF or a placebo (PL). Subjects followed a VLCD ($< 10\%$ total kcal from carbohydrate) for 12 weeks and consumed 3g/d of either SF or PL. Measured parameters were blood lipids, apolipoproteins (Apos), glucose, bodyweight, waist circumference, whole and regional body composition (DEXA), urinary ketones, and blood pressure. Dietary compliance was excellent. Because no significant differences ($P > 0.05$) were observed between groups in any parameter after 12 weeks, data were pooled for further analysis. At the end of the intervention, subjects had experienced reductions ($P < 0.001$) in plasma TG (from 117.7 ± 50.5 mg/dL to 72.2 ± 31.8) and glucose (from 93.1 ± 13.1 mg/dL to 89.2 ± 10.9 mg/dL), with increases ($P < 0.05$) in HDL-C (from 41.5 ± 11.1 mg/dL to 46.5 ± 12.9 mg/dL). The improvements in plasma lipids were associated with reductions in Apos C1 (-13.7%), E (-11.6%), and C3 (-21.3%). At 12 weeks, there was also a reduction ($P < 0.001$) in bodyweight (-7.5 ± 2.5 kg), percent body fat ($32.1 \pm 4.4\%$ to $28.0 \pm 5.8\%$), waist circumference (-8.0 ± 4.0 cm) and a reduction ($P < 0.01$) in systolic blood pressure (124.3 ± 10.1 mmHg to 116.7 ± 9.0 mmHg). At baseline, 11 subjects met the diagnostic criteria for metabolic syndrome (MetSyn). Cases of MetSyn were reduced to 3 by week 12. These results indicate that a VLCD is an appropriate dietary intervention for overweight men with the MetSyn.

THE SIGNIFICANCE OF APOE PHENOTYPE TO THE COMPOSITION, STRUCTURE AND BINDING OF VLDL AND LDL TO THE LDL RECEPTOR IN NORMO- AND HYPERTRIGLYCERIDEMIA

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

THE TERTIARY AND QUATERNARY STRUCTURE OF HUMAN APOLIPOPROTEIN E2, E3 AND E4 IN SOLUTION

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Three recombinant apoE isoforms fused with an amino-terminal extension of 43 amino acids were produced in a heterologous expression system in *E. coli*. By liquid chromatography, all three isoforms consisted of three major species with Stokes radii of 4.0, 5.0 and 6.6 nm, in contrast to plasma apoE3 (p-apoE3) which exists essentially as 6.6 nm tetramer. Sedimentation velocity confirmed the presence of monomers and dimers. The association schemes established by sedimentation equilibrium experiments corresponded to dimer-tetramer-octamer for r-apoE2, monomer-dimer-tetramer for r-apoE3 and monomer-dimer-tetramer-octamer for r-apoE4. Thus, each of the three recombinant isoforms exhibits a distinct self-association pattern which differs from that of p-apoE3. The apolipoprotein domain structure was mapped by limited proteolysis with trypsin, chymotrypsin, elastase, subtilisin and *Staphylococcus aureus* V8 protease. All five enzymes produced stable intermediates during the degradation of the three isoforms, as described for p-apoE3. The recombinant apoE isoforms, thus, consist of N- and C-terminal domains. The presence of the fusion peptide did not appear to alter the apolipoprotein tertiary organization. However, a 30 kDa amino-terminal fragment appeared during the degradation of the recombinant isoforms resulting from cleavage in the 273-278 region. This region, not accessible in p-apoE3, results from a different conformation of the C-terminal domain in the recombinant isoforms. A specific pattern for the r-apoE4 C-terminal domain was observed during the proteolysis. The region 230-260 in r-apoE4, in contrast to that of r-apoE3 and r-apoE2, was not accessible to proteases, probably due to the existence of a longer helix in this region of r-apoE4 stabilized by an interdomain interaction.

THE EFFECTS OF HYPERBARIC OXYGEN TREATMENT ON THE REGRESSION OF ATHEROSCLEROSIS IN RABBITS

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Background: General problem of circulatory system is the impaired function of the blood vessel system. The rabbits have tendency to rapidly develop severe hypercholesterolemia leading to premature atherosclerosis in response to dietary manipulation. **Objective:** In this study we have tried to establish the effect of hyperbaric oxygen (HBO) treatment, on the extent of diet-induced accumulation of lipid oxidation products in rabbit plasma and tissues, and the extent of progression and regression of atherosclerotic lesions in the rabbit aorta. **Material and Methods:** Animals were divided in three groups one control (n=7) and two experimental A and B groups (n (A group)=7 n (B group)=7). Both experimental groups have been submitted to cholesterol-rich diet, but only experimental group A have received HBO treatment (7 HBO treatments 2,5 ATA 70 min.). **Results:** As expected, cholesterol-rich diet induced sever hypercholesterolemia. HBO treatment had little or no effect on plasma cholesterol concentrations. We have also found, in the plasma of the experimental animal the substation reduce high density lipoprotein fraction. But significant reduction in the accumulation of cholesterol in the aortic segments, was noticed on the preparations taken from the animals from the group A compared to the similar preparations from the group B. In spite of the fact that HBO treatment did not have effect on the rate of plasma cholesterol decline, it significantly accelerated aortic lesion regression, as it has been shown on the histology tissue preparation. On the histology tissue preparation from the experimental group A, reduction in the remodeling process of the myocardium were also shown. **Conclusion:** On the basis of these result we can assume that repeated (even relatively short), exposure to HBO induces an antioxidant defense mechanism (or mechanisms), that is (are) responsible for retarding the development of atherosclerotic lesions.

HETEROZYGOUS MUTATION OF ATAXIA TELANGIECTASIA MUTATED GENE AGGRAVATES HYPERCHOLESTEROLEMIA IN APOE-DEFICIENT MICE

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Individuals with a heterozygous mutation at the ataxia-telangiectasia-mutated gene (*ATM*) may be predisposed to ischemic heart disease. This report examined for the first time the effect of a heterozygous *ATM* mutation (*ATM*^{+/−}) on plasma lipid levels and atherosclerosis intensity using *ATM*^{+/−}, *ATM*^{+/+} (wild-type), *ATM*^{+/−}/*LDLR*^{−/−} (low density lipoprotein receptor knockout), *ATM*^{+/−}/*LDLR*^{−/−}, *ATM*^{+/+}/*ApoE*^{−/−} (apolipoprotein E knockout) and *ATM*^{+/−}/*ApoE*^{−/−} mice. Our data demonstrated that the plasma cholesterol and triglyceride levels in *ATM*^{+/−} and *ATM*^{+/−}/*LDLR*^{−/−} mice were approximately the same as those in *ATM*^{+/+} and *ATM*^{+/+}/*LDLR*^{−/−} control mice, respectively. In contrast, the plasma cholesterol level was significantly higher in *ATM*^{+/−}/*ApoE*^{−/−} mice than in *ATM*^{+/+}/*ApoE*^{−/−} control mice. In addition, the *ATM*^{+/−}/*ApoE*^{−/−} mice showed higher plasma apolipoprotein B48 levels, slower clearance for plasma apolipoprotein B48-carrying lipoproteins and more advanced atherosclerotic lesions in the aorta when compared to the *ATM*^{+/+}/*ApoE*^{−/−} mice. These novel results suggest that the product of *ATM* is involved in an ApoE-independent pathway for catabolism of apolipoprotein B48-carrying remnants, and therefore superimposition of a heterozygous *ATM* mutation onto an *ApoE* deficiency background reduces the clearance of apolipoprotein B48-carrying lipoproteins from the blood circulation, and promotes the formation of atherosclerosis.

TRIGLYCERIDES TO HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO AS A PREDICTOR OF C-REACTIVE PROTEIN IN MILITARY PILOTS IN THE SERBIA AND MONTENEGRO

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Background: Relationship between C-reactive protein and features of metabolic syndrome (MS) in apparently healthy men is not fully elucidated. **Aim:** To assess the cross-sectional relationship between CRP and cardiovascular risk factors (age, total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), triglycerides (Tg), fasting glucose, glycosylated hemoglobin (HbA1c), blood pressure, smoking habit, waist circumference (WC), body mass index (BMI)) in military pilots on their regular annual medical control. **Methods and Results:** We studied 205 pilots (age 38+/-6 years) free of cardiovascular disease and diabetes mellitus. Plasma CRP was measured by immunonephelometry (Dade Behring) method. MS was defined according to NCEP ATPIII. The means of CRP concentrations in subjects grouped according to the presence of 0, 1, 2 and 3 or more features of the MS were 1.11, 1.89, 1.72 and 2.22 mg/L, respectively, (ANOVA p=0.023) with a statistically significant difference between those with and without MS (p=0.01). In simple regression analyses CRP not correlated with TC, LDL-c, HDL-c, BMI and blood pressure. In multiple regression analysis WC (β=0.411, p=0.000), Tg to HDL-c ratio (β=0.774, p=0.000), smoking habit (β=0.236, p=0.003) and Tg (β=0.471, p=0.027) were independent predictors of CRP. **Conclusions:** Our results suggest a cross-sectional independent correlation between features of metabolic syndrome, particularly waist circumference and triglycerides to HDL ratio and CRP in apparently healthy subjects. The lack of correlation of CRP with TC and LDL-c may suggest the possibility to determine CRP in order to identify high-risk subjects not identified with cholesterol screening.

HYPERTRIGLYCERIDEMIA IS ASSOCIATED WITH IMPAIRED ENDOTHELIAL-DEPENDENT VASODILATION AND MARKERS OF OXIDATIVE STRESS

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Aim: Comparison of subjects with high and low cholesterol levels in markers of endothelial dysfunction, intima-media thickness of common carotid artery (IMT) and oxidative stress.

Subjects and methods: 189 individuals were divided into quartiles according to their plasma triglycerides levels (TG). Subsequently the analysis was performed comparing the groups of the highest and the lowest quartile (mean age 54,1±4,18 vs. 54,1±4,63; p=0,973). Endothelium dependent vasodilation (EDV) was assessed on brachial artery using the method described by Celermajer. IMT was used as a marker of advanced atherosclerosis. Oxidative stress was measured using the levels of oxLDL, anti-oxLDL, MDA, F2-isoprostanes, AOPP and the levels of antioxidant vitamins (retinol and tocopherol).

Results: Patients in highest quartile of TG had significantly lower values of EDV (3,3±2,85 vs. 5,3±4,16; p=0,024), and higher IMT (0,68±0,173 vs. 0,62±0,175; p=0,035). Similarly, patients with hypertriglyceridemia showed significantly higher values of oxLDL (72,6±10,02 vs. 55,2±7,67; p=0,002) and AOPP (220,8±45,93 vs. 84,9±13,93; p=0,001). Higher plasma levels of retinol (0,73±0,084 vs. 0,63±0,065; p=0,0054), ferritin (169,1±57,98 vs. 104,2±47,53; p=0,015) and lower Tf/ferritin ratio (0,01±0,004 vs. 0,02±0,010; p=0,043) in hypertriglyceridemic subjects may reflect higher red meat consumption in these individuals.

Conclusions: Patients in highest triglycerides quartile showed impaired EDV and higher markers of oxidative stress.

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TRIGLYCERIDES, HDL CHOLESTEROL AND ACCUMULATION OF RISK FACTORS IN A PORTUGUESE SAMPLE POPULATION

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The importance of several lipidic and nonlipidic risks factors in the development of atherosclerosis and consequently of cardiovascular diseases (CVD) is very well known. Though, the prevention of these diseases increasingly relies on the individual evaluation of these risk factors and their accumulation to subsequent control of those which are modifiable. Besides LDL cholesterol it is evident that the predictive value of increased blood concentration of triglycerides (TG) especially when it coincide with low HDL-c have to be considered in assessing the CVD risk. So the authors decide to inquire this dyslipidemic type (high triglyceride, low HDL-c) and its accumulation with other risk factors and a population sample of the Lisbon area was considered. It consisted of 438 subjects, both sex, 30 to 80 years old. In these fasting subjects, triglycerides, total, and HDL cholesterol was quantified using automated laboratory methods, LDL cholesterol was calculated using Friedewald's formula, systolic and diastolic blood pressure (SBP and DPB) were measured and BMI and hip circumference ratio (WHR) calculated, smoking habits inquired.

Our results have shown according with triglycerides and HDL-c levels: higher percentages of individuals at risk for the risk factors considered: SBP, DBP, smoking, BMI and WHR in dyslipidemic subjects (TG ≥1.71 and HDL-c <1.03 mmol/l); statistically significant difference of these last risk factors in relation to normolipidemic subjects (TG <1.71 and HDL-c ≥1.03 mmol/l). The only exception to these findings was concerned with total and LDL cholesterol. On the other hand the number of CVD risk factors considered (one, two, three, and four) was always higher in dyslipidemic than in normolipidemic subjects.

In **conclusion** the risk factors considered, all modifiable, have to be better changed individually using therapeutic or educational measures to involve dietary and lifestyle habits.

DECREASED LIPOPROTEIN LIPASE ACTIVITY (LPL) AND INCREASED CONCENTRATIONS OF POSTPRANDIAL TRIGLYCERIDE-RICH LIPOPROTEINS (TRL) IN ELDERLY SURVIVORS OF MYOCARDIAL INFARCTION.

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Background: A low plasma concentration of HDL- cholesterol (HDL-C) is a strong and independent risk factor for coronary artery disease associated with increased postprandial concentrations of TRL. **Aims:** To investigate the metabolism of TRL in elderly patients with a previous history of myocardial infarction (MI) and healthy controls, and to determine the relationship in postprandial changes with lipoprotein lipase activity. **Methods:** A case-control study was performed in which 44 elderly (mean 74±5.4 yrs) MI patients and 43 healthy controls matched for age and gender were recruited. Each subject was given a standardized oral fat load (1g/kg body weight) and blood samples were collected every second hour for an 8-hour period. The LPL activity was determined before and after heparin administration (100 IU/kg body weight). **Results:** MI patients had significant lower HDL cholesterol than the controls (1.66±0.47 vs. 1.45±0.12, p=0.028). MI patients had significant higher incremental and total are under the curve (AUC_i and AUC respectively) for both triglycerides (p=0.040) and chylomicrons (p<0.001). Postheparin LPL activity was significant lower in MI patients compared to controls (87.41 ± 36.91 vs. 106.03 ± 29.03 IU/ml p= 0.014). AUC_i for serum triglycerides and chylomicrons were inversely correlated to postheparin LPL activity (p=0.05 and p=0.003 respectively). **Conclusions:** Elderly patients with a previous history of MI had increased levels of TRL in the postprandial state and reduced postheparin LPL activity. This study indicates that low fasting HDL-C may be a marker of increased postprandial concentrations of TRL and reduced LPL activity in elderly patients with MI.

GENDER AND AGE RELATED DIFFERENCES IN TREATMENT AND CONTROL OF CARDIOVASCULAR RISK FACTORS AMONG HIGH-RISK PATIENTS WITH ANGINA

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Dyslipidemic, hypertensive patients (N=48,863) were stratified by gender, age, and angina (n=2,502) /non-angina (n=46,358) status. Confidence intervals (CI=95%) indicated significant differences in treatment and cardiovascular risk factor control between sub-groups. More men than women had LDL-cholesterol <100mg/dL (CI=angina 43.93-43.96 vs. 34.42-34.49 / non-angina 32.42-32.43 vs. 17.24-17.25) and ≥100-<130mg/dL (CI=angina 32.11-32.14 vs. 35.10-35.17 / non-angina 53.85-53.86 vs. 32.43-32.44). More women than men had LDL-c ≥130mg/dL (CI=angina 27.67-27.72 vs. 23.91-23.92 / non-angina 38.69-38.70 vs. 35.38-35.39). Women were less likely than men to receive: statins (CI=angina 69.95-69.99 vs. 82.11-82.13 / non-angina 59.80-59.80 vs. 63.72-63.72); any anti-lipidemic medication at all (CI=angina 25.93-25.97 vs. 13.48-13.48 / non-angina 36.73-36.73 vs. 30.73-30.73), or to have current cholesterol measurements (CI=angina 56.82-56.88 vs. 34.54-34.56 / non-angina 45.77-45.77 vs. 39.75-39.75). Primary care providers treat high-risk patients relatively aggressively. However, opportunities to forestall cardiovascular disease may be missed in hypertensive, dyslipidemic women whose LDL-c is often not measured and controlled.

INTERMEDIATE DENSITY LIPOPROTEIN STIMULATES ALTERED EXPRESSION AND DISTRIBUTION OF VCAM-1

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Introduction: Endothelial cell dysfunction is characterised by an upregulation of membrane-bound glycoproteins termed cell adhesion molecules (CAM). CAMs are responsible for anchoring leukocytes to the vascular wall and facilitating the transmigration of adhered cells into the arterial intima during initiating stages of atherogenesis. Also, soluble forms of CAMs are regarded as a novel biomarker of cardiovascular risk.

Aim: The aim of this study was to examine the expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells following incubation with physiologically relevant concentrations of remnant lipoproteins, in the form of native- and oxidised- intermediate density lipoprotein (IDL).

Methods: Confluent primary human coronary artery endothelial cells (HCAECs) were starved of lipoproteins for 24 hours before incubation with IDL treatments. Cellular (c)VCAM-1 was quantified by Flow Cytometry and soluble levels assayed in the tissue culture medium, using commercially available monoclonal ELISA kits.

Results: Exposure of cells to native and oxidised IDL stimulated an increase in sVCAM-1. The levels of sVCAM-1 did not correlate with the expression of cVCAM-1; on the contrary, IDL treatments induced an alteration in the ratio of soluble to cellular VCAM-1. In general, a reduction in cVCAM-1 was accompanied by an increase in sVCAM-1 with increasing concentration and oxidative modification of IDL.

Conclusions: In conclusion, these findings indicate that *in vitro* physiological concentrations of IDL have a pro-atherogenic effect on HCAECs and stimulate changes in the expression and distribution of VCAM-1. These findings provide an insight into the mechanisms which link elevated remnant lipoproteins, endothelial dysfunction and premature atherosclerosis.

HYPERLIPIDEMIC SERUM CAN INDUCE A NOVEL MARKER (PC-CHOLESTEROL COMPLEX) OF FOAM CELL DEATH

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[Objective] Phosphatidylcholine (PC)-cholesterol (FC) complex structures, a unique atheroma related antigen recognizing by a novel monoclonal antibody **ASH1a/256C**. To elucidate possible mechanisms of expression of this antigen, the relationship between FC accumulation and several apoptotic events in cultured foam cell were investigated.

[Method] J774 cells incubated in the presence of hyperlipidemic sera or modified lipoproteins for 24 hr, then cultured up to 3-13 days. PC-FC complex were detected by immunofluorescent microscopy. UPR marker (CHOP, XBP-1) was detected by immunoblot analysis.

[Results & Discussion] Using human or WHHL rabbit hyperlipidemic sera and J774 cells, we succeeded to express PC-FC complex on the surface of FC-rich lipid droplets as a foam cell death marker. PC-FC complex could be formed only when the cells were incubated with hyperlipidemic sera or AcLDL, not VLDL, LDL. Moreover, VLDL enhances PC-FC complex formation by AcLDL; indeed, VLDL could not induce it by itself. Annexin-V positive cells were observed at day 3 induced by hyperlipidemic sera, whereas native LDL, HDL and VLDL failed to induce among FC-rich lipid droplets, PC-FC complex and Annexin-V binding structures. TUNEL positive cells were also observed in this foam cell culture at day 4 or later, whereas, no apoptotic cells were observed after treatment with **U18666A** in order to block cellular cholesterol transport. The UPR reaction marker, **CHOP** and **XBP-1** were also detected. The hydrolysis and rearrangement of cellular cholesterol take place in foam cells to form the antigen; there were significantly relationship between the PC-FC complex formation and foam cell death.

THE OXIDATION POTENTIAL OF VLDL, LDL AND HDL IN THE PRESENCE OF 5 DIFFERENT FLAVONOIDS

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Introduction: Epidemiological studies have demonstrated an inverse relationship between dietary intake of flavonoids and mortality from coronary heart disease (CHD). Oxidative modification of lipoproteins, a key early event in the development of atherosclerosis, can be inhibited by flavonoids. The mode of action of these compounds lies in their ability to chelate metal ions, scavenge free radicals and recycle other chain breaking antioxidants.

Aim: To assess if quercetin, myricetin, kampferol, luteolin and apigenin alter the susceptibility of VLDL, LDL and HDL to oxidation, and to assess if, when present in combination, they act synergistically.

Methods: Lipoproteins were isolated by rapid ultracentrifugation: VLDL and LDL 100,00 rpm for 1 hour; HDL 100,00 rpm for 2 hours, at 4°C. Lipoproteins were standardised for protein, and oxidised in the presence of copper II chloride. The antioxidant potential of each flavonoid was examined individually and in combination over the concentration ranges of 0→10 µM. Oxidation was monitored at 234 nm in a thermostatically controlled microplate reader, at 37°C.

Results: Examination of each of the individual antioxidants demonstrated that for all 3 lipoprotein classes, quercetin was the most potent antioxidant followed by luteolin, myricetin, kampferol and apigenin. We also demonstrated that in the presence of each of the 5 flavonoids, HDL was afforded the greatest protection followed by LDL and then VLDL. The antioxidant protection afforded to the lipoproteins during flavonoid combination demonstrated an additive effect and not a synergistic effect.

Conclusions: In vitro, flavonoids demonstrate varying antioxidant potentials and also target each of the 3 major lipoprotein classes with differing potencies.

HYPERHOMOCYSTEINEMIA IN CHRONIC HEART FAILURE

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Background. Hiperhomocysteinemia (HHcy) exerts numerous adverse biological effects on the vascular bed and myocardium. Patients with chronic heart failure (CHF) may be particularly susceptible to unfavorable effects of HHcy, but this has not been comprehensively investigated. The aim of this study was to assess the prevalence of HHcy (prospectively defined as plasma homocysteine [Hcy] level ≥ 14 µmol/L) in the unselected cohort of CHF patients, its clinical implications and pathophysiological links.

Methods and results. In 113 consecutive patients with CHF (81 men, age: 65 ± 11 years) mean plasma Hcy level was 13.2 ± 7.0 µmol/L, (range 2.3 – 50.0 µmol/L) and 41 (36%) patients had HHcy. Among clinical and metabolic parameters only advanced NYHA ($p=0.04$) and elevated serum uric acid ($p=0.0008$) predicted HHcy after correction in the multivariate analysis. HHcy was related to impaired survival (hazard ratio 4.0, 95% CI: 1.8-8.6, $p=0.0005$) also when adjusted for conventional risk predictors (all $p < 0.05$). In patients with HHcy 18-month survival was 63% (95% CI: 49-78%) as compared to 88% (95% CI: 80-95%) in those with normal Hcy levels ($p=0.002$). The second study confirmed an elevated Hcy levels in 38 CHF patients when compared with 30 age-, sex-matched healthy controls ($p<0.0001$) and demonstrated significant correlations between plasma Hcy and pro-inflammatory cytokines (interleukin[IL]-6: $r=0.25$, $p<0.05$ and tumor necrosis factor [TNF]-alpha: $r=0.43$, $p=0.0002$ and C-reactive protein (hsCRP) ($r=0.40$, $p=0.0009$).

Conclusion. HHcy commonly occurs among unselected CHF patients, is related to the disease severity, depicts generalized metabolic imbalance (evidenced by hiperuricaemia) and independently predicts poor prognosis. In particular, elevated Hcy level may reflect proinflammatory activation.

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

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Objective: Elevated levels of triglyceride-rich lipoproteins (TGRL) are a cardiovascular risk factor and have been shown to induce endothelial dysfunction; however the molecular mechanisms and the gene expression patterns underlying these effects are poorly understood. In the present work we investigated the effects of triglyceride-rich lipoproteins from hypertriglyceridemic and normotriglyceridemic subjects on endothelial function focussing on activation of intracellular pathways and gene expression in human endothelial cells. **Results:** A total of 53 subjects, 30 hypertriglyceridemic (TG levels $284,4 \pm 101,1$ mg/dL) and 23 normotriglyceridemic (TG levels $108,65 \pm 39,9$ mg/dL) were enrolled into the study. Human endothelial cells were incubated with TGRL isolated from hypertriglyceridemic (H-TGRL) and normotriglyceridemic (N-TGRL) subjects. Western blotting analysis and protein/DNA arrays showed that H-TGRL mainly activated p38MAPK, CREB and NF- κ B, AP-1 as well as Bm-3, CDP, NF-1, NFE1, NFE2. Total RNA was processed for cDNA microarray analysis. H-TGRL mainly induced the expression of adhesion molecules such as VCAM-1, PECAM-1, ELAM-1, P-selectin, chemotactic factors such as MCP-1, cytokines such as IL-6, receptors such as TLR-4 and CD40, and proteases such as PAI-1 and ADAMTS1. This profile was characteristic of H-TGRL as N-TGRL increased only the expression of VCAM-1, PECAM-1 and PAI-1. These findings were validated with quantitative real-time PCR and immunofluorescence studies. Furthermore, bioinformatic analysis and chromatin-immunoprecipitation (CHIP) studies confirmed that NF- κ B and CREB were mainly responsible for the effects observed. **Conclusion:** These findings confirm the involvement of TGRL from hypertriglyceridemic subjects in endothelial dysfunction via the induction of pro-inflammatory and pro-thrombotic responses.

THE ROLE OF TG AND HDL LEVELS ON LATTER THROMBOTIC STROKE IN GREEK POPULATION

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Epidemiological studies have demonstrated that increased Triglycerides (TG), and decreased HDL are associated with significant cardiovascular risk and are predisposing factors for coronary heart disease and stroke.

Purpose: The aim of this study was to investigate the exact impact of TG and HDL on patients with latter acute thrombotic or embolic stroke episode in Greek population.

Material and Methods: 847 inpatients aged 76 ± 9 years with latter acute thromboembolic stroke episodes, 455 females (F) and 392 males (M) were studied. Investigated TG and HDL and recorded the presence blood press (BP), and diabetes mellitus (DM). All patients belong to our Internal Medicine Clinic and results were analyzed in the same laboratory. The proportion of death was 17%. Statistical analysis was performed using a SPSS 11.0.

Results: TC 215 ± 60 , TG 129 ± 70 , HDL 46 ± 14 , and LDL 139 ± 46 . The lipid profile between F and M was similar without statistically significant. DM - TG: 148 ± 83 yes DM - 118 ± 58 no DM ($p=0,001$), DM - HDL: 45 ± 14 yes DM - 47 ± 14 no DM ($p=0,693$), BP - TG: 130 ± 72 yes BP - 127 ± 67 no BP ($p=0,336$), BP - HDL: 47 ± 14 yes BR - 45 ± 13 no BR ($p=0,306$). (All lipid values are mg/dL).

Conclusion: The study shows that in latter thromboembolic strokes the TG are increased statistically significant in patients with DM. In patients with BP was not difference statistically significant to HDL.

THE INTERACTION BETWEEN POSTPRANDIAL TRIGLYCERIDES, LIPOPROTEIN LIPASE (LPL) ACTIVITY AND APOLIPOPROTEIN C-I (APO C-I)

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Introduction: Postprandial accumulation of triglyceride-rich lipoproteins (TRL) may promote the development of atherosclerosis, and prolonged postprandial triglyceridemia is associated with increased carotid artery IMT. The amount of Apo C-I on TRL is associated with both coronary artery disease and degree of carotid atherosclerosis. **Objective:** To investigate the interactions between LPL activity, HDL cholesterol and Apo C-I in the postprandial metabolism of TRL. **Methods:** We recruited 95 persons, aged 56 to 80 years (47.5% men), from a population health survey. Blood samples were collected before and at 2-hours interval for 8 hours after a standard high fat meal. LPL activity was determined before and after heparin administration (100IU/kg). Apo C-I was measured in isolated VLDL by an enzymatic immunoassay. Incremental area under the curve (AUCi) for serum triglycerides (TG) and triglycerides in isolated chylomicrons (CM-TG) were assessed. **Results:** HDL cholesterol levels, AUCi TG and AUCi CM-TG were assessed across quartiles of LPL activity. HDL increased significantly with LPL activity (p for trend < 0.001). Both AUCi TG and AUCi CM-TG decreased significant with increasing LPL activity (p for trend 0.004 and 0.005 respectively). There was a significant linear trend for increase in AUCi TG and AUCi CM-TG across increasing quartiles of ApoC-I levels ($p < 0.001$). Similarly, LPL activity decreased significantly with increasing ApoC-I levels ($p=0.02$). **Conclusions:** Enrichment of TRL with Apo C-I, low LPL activity and delayed TRL clearance are interconnected and may display an unfavourable risk profile for atherosclerosis. The present findings may indicate that Apo C-I in TRL attenuates LPL activity and thereby promotes delayed clearance of postprandial TRL.

MINOR COMPONENTS OF VIRGIN OLIVE OIL AFFECT TRIGLYCERIDE-RICH LIPOPROTEIN HYDROLYSIS BUT NOT UPTAKE BY RAT HEPATOCYTES

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Both the TG molecular species and minor components of dietary oils might affect the hydrolysis and hepatic uptake of TRL remnants. As these particles have been shown to be atherogenic the rapid removal of these particles from blood is important. In the present study we evaluated the effect on lipid composition and TRL uptake by rat hepatocytes of dietary oils differing in TG molecular species composition and/or minor components: high-oleic sunflower (HOSO), virgin olive (VOO) and enriched virgin olive (EVO) oils, containing 1.1%, 1.2% and 2.4% of unsaponifiable matter, respectively. TRL were obtained from volunteers 4h after the intake of meals enriched in the oils and the lipid composition was determined. TRL were incubated with rat primary hepatocytes for appropriate times. TRL formed after the intake of VOO contained a lower concentration of TG. In addition, VOO-derived TRL were incorporated more slowly to hepatocytes, as observed by fluorescence microscopy and the mRNA expression of low-density-lipoprotein receptor (LDLr) and the protein related to the LDLr (LRP) was also lower compared to the other dietary oils. Therefore, we conclude that the unsaponifiable fraction of virgin olive oil increases mRNA expression for lipoprotein receptors but does not increase their uptake, which suggests that posttranscriptional regulation by components of the unsaponifiable fraction may be involved. **Acknowledgements:** This work was supported by grants from the CICYT (AGL2002-00195 ALI) and Juan de la Cierva contract to JSP.

IL-1 β AFFECTS LIPID METABOLISM IN MACROPHAGE-DERIVED FOAM CELLS

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In fibroblasts, the pro-inflammatory cytokine interleukin-1 β (IL-1 β) decreases intracellular total cholesterol levels through a complex combination of decreased synthesis, increased esterification and increased efflux of the lipid. The current study assessed the hypothesis that IL-1 β has similar effects on macrophage/foam cell cholesterol metabolism.

Differentiated primary human macrophages or THP-1 cells were lipid loaded with aggregated low-density lipoprotein (agLDL) or very low-density lipoprotein (VLDL) to increase intracellular lipid levels (i.e. create foam cells). Cells were then incubated with an increasing concentration of IL-1 β (0 – 5000 pg/ml). In both primary macrophages and THP-1 cells exogenous IL-1 β concentrations correlates positively with intracellular cholesterol levels. The same positive correlation is even stronger with intracellular triglyceride levels. Experiments exchanging IL-1 β with tumor necrosis factor alpha (TNF- α) gave similar results.

Comparisons of lipid values directly after incubations with lipoprotein with lipid values after lipoprotein and IL-1 β treatment suggest that increased cellular lipid content after incubation with the pro-inflammatory cytokine is the result of decreased intracellular lipid catabolism and/or efflux.

Findings in human mesangial cells (HMC), where IL-1 β increase intracellular cholesterol levels and reduce adenosine triphosphate (ATP) binding cassette A1 (ABCA1) gene expression, suggests impaired cholesterol efflux in those cells. As endogenous apoE stimulates cholesterol efflux from macrophages we measured apolipoprotein E (apoE) secretion in the two model systems. In primary macrophages there is a decreased apoE secretion consistent with increased intracellular lipid levels. In THP-1 cells there is a small, but significant increase when cells are loaded with VLDL.

All in all, it appears that pro-inflammatory cytokines tested in our experiments slow down macrophages capacity to catabolize intracellular lipids, resulting in enhanced foam cell formation. If present *in-vivo*, such mechanisms would further enhance the pro-atherogenic properties of IL-1 β and TNF- α , accelerating foam cell accumulation in plaques.

ADIPOPHILIN INCREASES TRIGLYCERIDES STORAGE IN HUMAN MACROPHAGES POTENTIALLY THROUGH A REDUCTION OF FATTY ACIDS β -OXIDATION

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In this study, we investigated the role of adipophilin on cellular lipid accumulation and lipid droplet formation in THP-1 macrophages. In the presence of AcLDL, THP-1 cells infected with an adenovirus expressing human adipophilin (Ad.CMV.adipophilin) showed a 31% increase in TG content and a greater number of lipid droplets compared to control, Ad.CMV.GFP-infected cells. Similar results were obtained when cells were incubated in the presence of VLDL, except for a dramatic increase of TG in both Ad.CMV.adipophilin-infected cells and in control cells. Indeed, incubation of macrophages with VLDL showed a significant stimulation of adipophilin protein levels in a dose-dependent manner. These changes were accompanied by an increased cellular content of lipid droplets. In contrast, inhibition of adipophilin expression in THP-1 macrophages, using siRNA prevented lipid droplet formation. Using inhibitors of fatty acid oxidation and acyl coA synthetase, results were obtained which suggest that adipophilin may inhibit fatty acid oxidation and channel long chain fatty acids into TG synthesis. In conclusion, adipophilin expression in THP-1 macrophages altered the cellular content of different lipids and enhanced the size of lipid droplets, consistent with a role for adipophilin in human foam cell formation.

ASSOCIATIONS BETWEEN DIFFERENT LEUCOCYTE COUNTS AND THE ACUTE MYOCARDIAL INFARCTION

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Several reports suggested that certain leucocyte count components such as neutrophils, are particularly relevant to atherosclerosis and can predict increased risks of coronary heart disease (CHD) such as acute myocardial infarction (AMI).

We aimed to assess potential associations between different leucocyte counts, taken of the vein blood and determined by standard methods in the first few hours after the AMI. We were included 230 middle aged (59.87 \pm 13 years old), mostly males (66.5%) and mostly obese (average BMI of 32.09 kg/m²) patients with ST-elevation acute myocardial infarction (STEMI).

Out of the main risk factors for obtaining a CHD in the group in which the research was performed, smoking was represented in the highest percentage (70.9%) arterial hypertension (58.3 %) and diabetes (46.1%), while a positive anamnesis for and existing hyperlipidemia was found at 35.7% of the patients. The follow up of the electrocardiograms of the patients with STEMI, the follow up of the echocardiograph tracking, high values of the markers of the myocardial necrosis and the markers of the inflammation (higher leucocyte count, sedimentation rate and fibrinogen level) and coagulation status, confirmed the diagnosis, at the same time, in favour of an extensive myocardial necrosis.

Our results provides assessment of specific leucocyte count in AMI, additional prospective data will be needed to resolve whether neutrophil count are much stronger predictors of CHD risk than other components. Identifying the triggers for inflammation and unraveling the details of inflammatory pathways may eventually furnish new therapeutic targets.

EVIDENCE FOR ELEVATED TRIGLYCERIDES AND LOW HDL CHOLESTEROL AS THE MAIN LIPID RISK FACTORS FOR DIABETIC ATHEROSCLEROSIS

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Background: There is a paucity of longitudinal data on the impact of serum lipids on the future incidence of vascular events among the high risk cohort of coronary patients with diabetes. **Methods:** We enrolled 750 consecutive patients undergoing coronary angiography for the evaluation of coronary artery disease (CAD). At baseline, serum lipids were measured, and the incidence of vascular events was recorded over 4 years. **Results:** From our coronary patients, 272 had normal fasting glucose <5.6 mmol/l (NFG), 314 impaired fasting glucose \geq 5.6 mmol/l (IFG), and 164 type 2 diabetes (T2DM). The incidence of vascular events significantly (p <0.001) increased from subjects with NFG (14.7%) over patients with IFG (19.4%) to patients with T2DM (30.5%). Factor analysis revealed two factors in the lipid profiles of our patients: triglycerides, HDL cholesterol, apolipoprotein A1, and LDL particle diameter loaded high on an HDL-related factor; and total cholesterol, LDL cholesterol, and apolipoprotein B loaded high on an LDL-related factor. The HDL-related factor (p <0.001) but not the LDL-related factor increased significantly from subjects with NFG over patients with IFG to patients with T2DM. In patients with T2DM, the HDL-related factor (OR 0.71 [0.51-0.97]; p = 0.032), but not the LDL-related factor (OR 1.16 [0.90-1.15]; p = 0.261) significantly predicted vascular events. **Conclusions:** The high triglyceride / low HDL pattern is associated with the degree of hyperglycemia and significantly predicts future vascular events among coronary patients with T2DM; it is thus the main lipid risk factor for vascular events in diabetic coronary patients.

EVALUATION OF EZETIMIBE/SIMVASTATIN VERSUS ATORVASTATIN ON ATHEROGENIC RATIOS OF NON-HDL-C/HDL-C AND APO B/APO A-I

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The on-treatment ratio of Apo B/Apo A-I has been recognized as a better indicator of the adequacy of reduction in atherogenic particles than LDL-C. Apo B/Apo A-I is considered the best measurement of balance between proatherogenic particles, which all contain one molecule of Apo B, and antiatherogenic particles, as Apo A-I is the major apolipoprotein in HDL-C. Apo B/Apo A-I is highly predictive of CHD risk in some epidemiological studies and in some clinical trials with lipid lowering statin therapy. In addition, this ratio correlates well with the more practical and easily calculated non-HDL-C/HDL-C ratio. In a population of 1902 patients randomized 1:1:1:1:1:1 to 8 arms (ezetimibe/simvastatin [E/S]10/10, 10/20, 10/40 and 10/80 mg and atorvastatin [A]10, 20, 40, and 80 mg), we evaluated efficacy of the combination product of E/S relative to A in percent change improvement of Apo B/Apo A-I and non-HDL-C/HDL-C ratios at four milligram-equivalent statin dose comparisons (10, 20, 40, 80 mg), and averaged across dose ranges after 6 weeks of treatment, by total population and by baseline TG level (<200, ≥200 mg/dL). For the total population, E/S demonstrated significantly greater improvement when averaged across dose ranges in Apo B/Apo A-I ratios (E/S: -45.0%, A: -38.7%; p<0.001) and non-HDL-C/HDL-C ratios (E/S: -52.0%, A: -43.8%; p<0.001) and at all milligram-equivalent statin doses, when compared with atorvastatin. Treatment effects were consistent across baseline TG subgroups. Ezetimibe/simvastatin provides greater efficacy to atorvastatin in improvement of Apo B/Apo A-I and non-HDL-C/HDL-C ratios after 6 weeks of treatment in patients with hypercholesterolemia, suggesting significant reductions in atherogenic particles in addition to LDL-C.

SAFETY OF ATORVASTATIN IN ELDERLY PATIENT POPULATION

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CV disease is the leading cause of death in the US. Of those who die of CHD and/or experience a stroke, 85% are ≥ 65 yrs. Sub-analyses suggest that statins benefit pts ≥ 65 yrs similarly as younger pts. Elderly may not receive evidence-based therapies, like statins, because of safety concerns. **Method:** This was an age-defined subgroup analysis of safety data in pts ≥ 65yrs from 50 randomized atorvastatin (Atv) trials completed by Sept 15, '04. The analysis included treatment-associated adverse events (AEs), serious AEs and musculoskeletal, hepatic and renal AEs in Atv 10-80 mg dose range and placebo (Pbo).

Results: A total of 5924 pts ≥65 yrs at the time of study enrollment were categorized in Atv 10mg (n=2042), 20mg (n=667), 40mg (n=522), 80mg (n=1698) and Pbo (n=995). Overall AE profiles for all Atv groups and Pbo were similar. Most frequent treatment-associated AEs were related to the digestive system (<7.5% all groups). Serious AEs were rare and seldom led to withdrawal. Pts with persistent elevation of LFTs (>3xULN) were 2(0.2%), 3(0.2%), 0, 1(0.2%), and 7(0.4%), in Pbo, Atv 10mg, 20mg, 40mg, and 80mg, resp. Persistent CPK elevations (>10xULN) were not observed in any treatment group. The incidence of treatment-associated myalgia was low. Hematuria was rare (1 pt in Pbo and Atv 80mg). There were no cases of treatment-related albuminuria and no cases of rhabdomyolysis.

Conclusion: The overall incidence of AE in Atv-treated elderly did not increase with dose and was similar to that observed with Pbo. The incidence of LFT elevations (>3xULN) was slightly higher in 80mg group, and specific musculoskeletal and hepatic AEs were rare. The results of this analysis supports the positive benefit to risk profile of atorvastatin and should be taken in consideration when managing the CV risk in the elderly.

PLEIOTROPIC EFFECTS OF LONG-TERM ATORVASTATIN TREATMENT IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background. Hyperlipidemia is a major risk factor for atherosclerosis, and several large scale trials demonstrate that cholesterol lowering therapy with statins reduces coronary event rates in both primary and secondary prevention. Paradoxes revealed by these trials, however, raise the possibility that statins may have effects that go beyond simple lipid reduction. **The aim** of the study was to estimate the effects of atorvastatin (lipimar) on anti-inflammatory markers (high sensitive C-reactive protein (hs-CRP) and fibrinogen) and endothelial function in patients (pts) with stable coronary artery disease (CAD). **Material and methods.** This was a randomized, double-blind placebo controlled trial of atorvastatin (20 mg) administered once daily. Pts were randomly assigned to one two treatment groups receiving either atorvastatin 20 mg daily (n=50) or placebo (n=50). Background therapy was similar for groups. EDRF release test was used to assess flow-mediated endothelium-dependent vasodilatation (FMD) of the brachial artery in response to reactive hyperemia. Plasma nitrite/ nitrate (NOx) levels were measured as an indirect index of nitric oxide (NO) production in vivo using HPLC. Duration of the study was 1 year. **Results.** At 1 year in the atorvastatin group, the average percent changes from baseline were as follows: total cholesterol - 31.2 %, LDL cholesterol -41.6%, TG - 25.09% (all P<0.001), HDL cholesterol +14.39% (P<0.05), Hs-CRP - 24, 1% (P<0.001), fibrinogen - 30.12% (P<0.001), FMD +66,3 % (P<0.001), NOx - 34.7% (P<0.001). Atorvastatin therapy has been shown to reduce significantly the incidence of total cardiovascular events, (12 vs 30 events). **Conclusion** The ensemble of data summarized above suggests that: atorvastatin has mark anti-inflammatory activity and significantly improved endothelial function in pts with CAD, i.e. it has pleiotropic effects.

EVALUATION OF THE SAFETY AND THE LIPID LOWERING EFFECTS OF TWO WEEKS ROSUVASTATIN TREATMENT IN PATIENTS WITH CORONARY HEART DISEASE

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Rosuvastatin is a new 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG – CoA) inhibitor. The aim of the study was to evaluate the efficacy and safety of two weeks rosuvastatin treatment in patients with hypercholesterolemia and coronary heart disease (CHD).

Methods: We studied 20 patients with stable CHD, symptomatic or mild to moderate angina and total cholesterol of at least 230mg/dl, low density lipoprotein cholesterol (LDL-C) – 155, 5 mg/dl, and serum level of triglycerides of less than 370mg/dl. Pts mean age was 56 ± 3, 5 years. Pts were randomly assigned to receive rosuvastatin (10mg/dl) or placebo. Background therapy was similar for group.

Results: after two weeks treatment in the rosuvastatin group the average percent changes from base line, were as follows: total cholesterol-33,6 % (P<0,001), LDL-C-45,91% (P<0,0001), high density lipoprotein cholesterol + 12,24%, (P<0,001), triglycerides-31,83% (P<0,001). In the placebo groups, the corresponding changes were: +1%, +2%, +2%. After two weeks rosuvastatin treatment the number of effort of attacks per day decreased by 78,9 % (P<0, 0001) and the consumption of nitroglycerine by 75, 8%, (P<0, 0001). Rosuvastatin has been shown to be highly effective in reducing LDL-C, and rising HDL-C in a wide range of patients. Therapy with Rosuvastatin was well tolerated. Adverse events occurred with similar frequency in the groups.

Conclusion: Rosuvastatin is the most effective of the current statins in enabling the majority of patients to achieve the latest goals for LDL-C at the usual starting dose and effective agent for the prevention of current ischemic events.

BENEFICIAL EFFECTS OF SIMVASTATIN (VASILIP) THERAPY ON ENDOTHELIAL FUNCTION AND INFLAMMATORY MARKERS IN PATIENTS WITH CORONARY HEART DISEASE (CHD)

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The study population consisted of 78 outpatients with CHD who received Simvastatin (Vasilip, KRKA, Slovenia) for 1 year. At the end of the follow-up mean dose of the drug was $19,1 \pm 2,3$ mg/d. Endothelial function assessed by brachial artery flow-mediated endothelium-dependent vasodilation (FMD) and plasma NO concentration was impaired prior to Vasilip treatment. At the end of the supervised period there was highly significant increase of FMD from $5,3 \pm 0,8\%$ to $8,4 \pm 0,44\%$ ($P < 0,01$). Enhanced thickness of carotid arteries intima-media complex decreased from $1,3 \pm 0,21$ mm to $1,1 \pm 0,04$ mm ($P < 0,01$). In 16 patients we observed remodelling-stabilization of atherosclerotic plaques. Carotid arteries occlusion degree reduced from $37,5 \pm 3,4\%$ to $20,2 \pm 3,4\%$ ($P < 0,001$). On the background of Vasilip therapy there was statistically highly significant increase in plasma NO levels to its normal mean value (from $11,2 \pm 1,8$ μ mol/l to $22,9 \pm 2,7$ μ mol/l). Examination of inflammatory markers showed substantial reductions in their blood concentrations. Compared with baseline ($3,8 \pm 0,65$ mg/dl and $6,9 \pm 1,1$ g/l) changes in mean plasma levels of C-reactive protein and fibrinogen were statistically significant as well ($1,7 \pm 0,56$ mg/dl and $2,9 \pm 0,3$ g/l, $P < 0,001$ and $P < 0,01$ respectively). So, Vasilip exhibited beneficial effects on endothelial function, carotid arteries atherosclerosis and inflammation. Due to obtained results we recommend the use of Vasilip therapy for the secondary prevention of coronary atherosclerosis.

TREATMENT WITH COENZYME Q10 AND SIMVASTATIN IN PATIENTS FOLLOWING MYOCARDIAL REVASCUARIZATION

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Despite the significant achievements of myocardial revascularization procedures—CABG and PCI, one of the clinical manifestations of atherosclerosis—CHD is the major cause of morbidity and mortality in the world. So, therapeutic interventions directed to the delay of the progression of coronary atherosclerosis remain still actual. It is natural that even complete and successful myocardial revascularization does not prevent the progression of atherosclerosis. Just on the contrary, CABG and PCI may induce inflammation, endothelial dysfunction, oxidative stress, which are some of the reasons of initial mechanism of restenosis or new stenosis in native coronary arteries as well as in bypass grafts. Consequently the goal of our trial was to estimate therapy with antioxidant coenzyme Q10 60mg/d ($n=32$) and its combination with simvastatin (zocor) 10mg/d ($n=32$) for an 8-week period. 34 patients had undergone CABG and 28-PCI. CoQ had a beneficial effect on HDL-C. It increased by 22% ($p < 0,01$). This effect was more expressed in combination with statin therapy. In this group antiatherogenic fraction of lipid profile increased by 28% ($p < 0,001$). The target levels of HDL-C were attained in 81% of cases. From 32 patients it increased in 31 cases. After 2-month study period in patients with CoQ treatment there was tendency to normalization of mean nitric oxide level. In contrast, in group with combination therapy there was statistically highly significant increase in NO to its normal mean value. So, the short-term treatment with CoQ demonstrated its potential independent role in positive modification of antiatherogenic fraction of lipid profile. Unlike simvastatin therapy CoQ is not able to normalize plasma NO levels. Taking into consideration that statins deplete the body of an antioxidant CoQ with consequent danger of heart failure and myopathy, and the obtained results of this study, we support the use of CoQ in combination with statins. Suggested attractive approach may be beneficial in preventing of further development of coronary atherosclerosis in all CHD patients with or without myocardial revascularization.

EFFECTS OF ARTERODIET ON BLOOD HDL-C AND LEVEL OF NITRIC OXIDE IN PATIENTS WITH CORONARY HEART DISEASE

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Patients with CHD are defined as the highest priority for preventive cardiology. Secondary prevention of CHD has focused on lowering LDL fraction of blood lipids not only because of the strong epidemiological evidence linking LDL to CHD, but also because pharmacological interventions have made it a highly modifiable risk factor. In contrast, although there is compelling epidemiological evidence that low plasma level of HDL-C is powerful independent risk factor for CHD. Besides there is growing evidence that endothelial dysfunction, is independent coronary risk factor as well. Arterodiet („Yves Ponroy”, France) is a dietary supplement consisting of natural active substances: polyunsaturated fatty acids, garlic powder, anthocyanosides and vitamin E. The goal of the present trial was to study the influence of arterodiet on HDL-C and endothelial function in patients with established CHD. The study population consisted of 47 outpatients. Beneficial effects of arterodiet was particularly expressed in association with antiatherogenic fraction of lipid profile. During 60 days of therapy there was statistically highly significant change in HDL-C. In parallel with increasing of antiatherogenic fraction there was substantial improvement in atherogenic ratio, which is widely used as an indicator of risk of future cardiovascular disease. In the beginning of the investigation patients had impaired endothelial function, which was estimated by examination of plasma nitric oxide concentrations. On the background of arterodiet therapy there was 31% increase of the mean plasma NO level ($p < 0,01$). Obtained data indicate that arterodiet may reverse endothelial dysfunction. According to the results of the study dietary supplement arterodiet may be used in secondary coronary prevention.

EFFECTS OF DIETARY PHYTIC ACID ON THE SERUM LIPID LEVELS IN AGED MICE

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Aging is a multi-factorial phenomenon. There are several clinical conditions directly related to the lipid metabolism that induces hypertriglyceridemia, hypercholesterolemia and cardiovascular disease during aging. There is a large body of evidence showing that triglyceride, cholesterol and LDL-cholesterol are among the major risk factors for cardiovascular disease, while HDL-cholesterol level is inversely correlated with such a risk. Attention has been focused on possible intervention strategies to control the levels of serum lipid, such as triglyceride, total cholesterol, LDL- or HDL-cholesterol. Phytic acid is a plant component which exists in most grains and legumes, the main source of the calorie intake for the old, and much research has been done on the antioxidant and antinutrient effects of phytic acid in the growing animal model. But its effect on the lipid profiles in the aged model has not been evaluated yet. The study was carried out to investigate the effect of phytic acid on the lipid levels in the serum of aged mice. Forty aged ICR male mice were fed with purified diets supplemented with 0 (P0), 0.5 (P5), 1.0 (P10), and 1.5 % (P15) sodium phytate for 12 weeks. Diet intake, body and organ weights, and contents of serum and fecal lipid profiles were measured. There were no significant differences in diet intake, body weight, organ weight and serum total cholesterol levels among experimental groups. The concentrations of the serum triglyceride and LDL-cholesterol were lower in the groups fed phytate diets than the P0 group. Serum HDL-cholesterol levels of the groups fed phytate diets were higher than the P0 group. The contents of fecal total lipid and total cholesterol were higher in the P10 and the P15 groups. These results suggested that phytic acid affect the serum lipid levels in aged mice by increasing their fecal lipid levels.

A NEW GENERATION OF ACTIVE COMPOUNDS FOR DYSLIPIDEMIA LOWERING BOTH CHOLESTEROL AND TRIGLYCERIDES - NUTRISEQUENCE TC

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Current recommendations focus on the decrease of LDL-cholesterol which is considered as a major element in the primary and secondary prevention of cardiovascular diseases. The metabolism of lipids is a complex biological system. Bioresearch & Partners chose to approach this syndrome non-medicinally opening up the possibility of effective supplementary therapies. The « micronutrisquence » developed by Bioresearch & Partners is a new way to regulate the TC and TG by a nutritional action of which the strategy is to participate at the restoration and the strengthening of the homeostasis of the lipid metabolism. The result is to reduce the levels of TC and TG to the physiological norms in more than 80% of the population. The ingredients are all nutritional and are sequenced for a better target. Method: The trial aimed to measure, for patients in monotherapy in open study, the decrease of TC and LDL-C, as well as the decrease of TG. The studied population presents, an excess of TC $\geq 5,93$ mmol/l, of LDL-C $\geq 3,87$ mmol/l and/or TG $\geq 1,71$ mmol/l. Period: 12-weeks. The population treated by lipid-lowering agents or complemented by fish oil or stanols was excluded. The population divided in 2 groups according to the type of hypercholesterolemia (combined or not). Results: In the population studied: 104 adults mean age: 60.7, presenting at D0 a TC average level 7,09 mmol/l and LDL-C 4,87 mmol/l, the results show a average decrease: TC 18% (5,77mmol/l), LDL-C 24% (3,63mmol/l) in 80 % of the cases (84 cases). In the group presenting combined hyperlipidemia (29 cases) with a TG average level at D0 of 2,76mmol/l, 82% (24 cases) record a TG average decrease of 35% (1,82mmol/l). "Nutrisquence TC" offers a new generation of product which has its own mode of action, totally compatible with all other therapeutic modes and works on both CT and TG. It can be used in monotherapy, in primary prevention alone, or in association. It is an innovative nutritional option which can be used in tablet or integrated into food, thus making easier the patient's acceptance and compliance.

ATORVASTATIN AND ITS EFFECTS ON VCAM-1, ICAM-1 AND ENDOGLIN ENDOTHELIAL EXPRESSION IN APOE-KNOCKOUT MICE

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The aim of this study was to detect and quantify the changes of endothelial expression of VCAM-1, ICAM-1 and endoglin after the administration of atorvastatin in ApoE-knockout mice. Male apoE^{-/-} mice were randomly divided into four groups. The control group (short-term) of animals was fed with the standard laboratory diet for 9 weeks and the long-term control group for 16 weeks. In both atorvastatin treated groups, atorvastatin was added to the chow diet at the dosage of 10 mg/kg per day for the last 4 or 8 weeks respectively, before the euthanasia. The results of the biochemical analysis showed mild increase in total cholesterol after 4 weeks administration of atorvastatin, but surprisingly significant increase after 8 weeks in compare to the short-term or long-term control group, respectively. Stereological analysis of VCAM-1, ICAM-1 and endoglin immunohistochemical staining revealed significant decrease in endothelial expression after 4 weeks of atorvastatin treatment but surprisingly strong increase in the expression of all markers in 8 weeks atorvastatin treated animals compared to the control groups. In conclusion we found that endothelial expression of VCAM-1 and endoglin is decreased by the pleiotropic effects of statins, but these effects can be over lapped by the strong hypercholesterolemia.

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THE EFFECT OF STATIN THERAPY AT RECURRENT CORONARY EVENTS IN PATIENTS FOLLOWING CORONARY STENT IMPLANTATION

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Objectives: We sought to investigate whether statin therapy affects the association between preprocedural C-reactive protein (CRP) levels and the risk for recurrent coronary events in patients undergoing coronary stent implantation.

Background: Low-grade inflammation as detected by elevated CRP levels predicts the risk of recurrent coronary events. The effect of inflammation on coronary risk may be attenuated by statin therapy.

Methods: We investigated a potential interrelation among statin therapy, serum evidence of inflammation, and the risk for recurrent coronary events in 84 consecutive patients undergoing coronary stent implantation. Patients were grouped according to the median CRP level (0,6 mg/dl) and to the presence of statin therapy.

Results: A primary combined end point event occurred significantly more frequently in patients with elevated CRP levels without statin therapy. Importantly, in the presence of statin therapy, the RR for recurrent events was significantly reduced in the patients with elevated CRP levels to about the same degree as in patients with CRP levels below 0,6mg-dl and who did not receive statin therapy.

Conclusion: Statin therapy significantly attenuates the increased risk for major adverse cardiac events in patients with elevated CRP levels undergoing coronary stent implantation, suggesting that statin therapy interferes with the detrimental effects of inflammation on accelerated atherosclerotic disease progression following coronary stenting.

STATINS INFLUENCE ON THE DYNAMICS OF HDL-C AND TG LEVEL IN HELICOBACTERY PYLORI AND CHLAMIDIA PNEUMONIAE INFECTED CHD PATIENTS

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The aim of this work is to study the dynamics of HDL-C and TG exchange parameters in Helicobacter Pylori (HP) and Chlamidia Pneumoniae (CP) infected CHD patients after eradication treatment with antibiotics and statins.

Materials and Methods: We examined CHD patients, among them: 46 with HP (confirmed by serologic diagnostic, histologically and by urease test) and 32 CP seropositive (more, than 1:32) patients.

For each patients Total Cholesterol (TC), Tryglicerides (TG), High Density Lipoproteins Cholesterol (HDL-C), Low Density Lipoproteins Cholesterol (LDL Cholesterol)-using Spectrophotometer, before and after 2 months therapy were performed. HP infected patients by accepted triple therapy (7 days) and CP infected patients by Azytromicine were treated during 2 months. Among them 22 HP patients and 18 CP patients 2 months atorvastatine were received.

Results and conclusion: of investigation show that infected patients after 2 months atorvastatine and eradication therapy had amelioration of clinical symptoms and lipid exchange parameters (for example HDL-C levels increase at 14% (P<0,01) and TG level decrease at 26% (P<0,01)), than patients with only after eradication therapy (for example HDL-C increase at 9% and TG levels decrease at 12% (P<0,05)).

Aggravation of atherosclerosis greatly depends of infection. The infected CHD patients will be treated with statins (atorvastatine) under eradication therapy.

AN ANALYSIS OF C-REACTIVE PROTEIN AND THE EFFICACY OF EZETIMIBE ADDED TO STATIN THERAPY

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High sensitivity C-reactive protein (CRP) is associated with increased risk for CHD. We examined the efficacy of ezetimibe 10 mg (EZE) vs. placebo (pbo) added to a stable dose of statin in modifying serum CRP levels. The Ezetimibe Add-On to Statin for Effectiveness (EASE) trial, a randomized, double-blind, 6-wk study, examined effectiveness and safety of EZE added to a stable dose of statin (N=3030; LDL-C above NCEP ATP III goals). Patients were randomized 2:1 to EZE or pbo. Baseline CRP levels were similar across treatment groups. ANOVA models were used to compare treatment groups using rank transformed values based on Tukey normal scores. EZE+statin significantly improved CRP from baseline (p<0.001) compared to pbo+statin (Table 1); the treatment effect was consistent across baseline CRP levels (treatment by CRP level interaction, p=0.572). Adding EZE provided additional improvements in CRP to statin-treated baseline values.

Table 1. Median Baseline, Postbaseline, and % Changes in CRP in MITT Population and by Baseline CRP Level

Patient pop.	Placebo Plus Statin				Ezetimibe Plus Statin			
	N	Baseline (mg/L)	At 6 Wks	% Change	N	Baseline (mg/L)	At 6 Wks	% Change
MITT	823	2.5	2.6	0.0	1656	2.4	2.0	-12.3*
Baseline CRP Level (mg/dL)								
<1.0	147	0.6	0.8	28.6	360	0.6	0.6	0.0†
1 to 3	328	1.7	1.8	0.0	588	1.8	1.5	-10.3†
>3.0	348	5.6	4.9	-16.4	708	5.8	4.7	-21.2†

*p<0.001 (percentage change EZE+statin compared with placebo+statin)

†Treatment effect was consistent across baseline CRP levels

ACTIVATION OF OVEREXPRESSED PPARγ INDUCED FOAM CELL LIKE CHANGES IN RAT VSMC

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The formation and accumulation of foam cells in the vessel wall is one of the hallmarks of the developing atherosclerosis. Although there has been studies showed evidence of VSMC derived foam cells, the understanding of mechanism of lipid uptake in SMCs is still very limited. We employed adenoviral gene delivery system to overexpress PPARγ in rVSMCs. When the cells were stimulated with synthetic PPARγ ligand, lipid accumulation and morphological changes of VSMC were evident. Also a drastic increase in mRNA of adipocyte differentiation marker genes and CD36 were observed suggesting the induction of PPARγ expression and its subsequent activation precede the scavenger receptor CD36 activation in the process of atherosclerosis and might be one of the crucial events in VSMC derived foam cell formation. This study demonstrated that PPARγ activation can induce transformation of VSMCs to foam cell like cells with lipid accumulation and up-regulated adipogenesis/lipid metabolism related gene expressions.

EZETIMIBE ADDED TO STATIN THERAPY REDUCES LDL-C, TG AND NON-HDL-C AND IMPROVES HDL-C IN PATIENTS WITH DIABETES AND METABOLIC SYNDROME

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Patients with diabetes (DM) and metabolic syndrome (MS) often exhibit dyslipidemia with low levels of HDL-C and elevated levels of triglyceride (TG) and non-HDL-C, despite normal or slightly elevated LDL-C levels. In the Ezetimibe Add-On to Statin Therapy for Effectiveness (EASE) trial, a randomized (2:1), controlled study (n=3030) of ezetimibe (EZE) vs. placebo added to a stable dose of statin, a third (38.4%) of the patients had DM, while 60.1% had MS (≥3 NCEP ATP III criteria). Another subset (24.6%) had metabolic dyslipidemia (MD) (low HDL-C, elevated TG and at least 1 other characteristic of MS). After 6 wks of treatment, in all three subgroups, adding EZE to ongoing statin therapy significantly improved multiple measures (Table 1), including total-C, LDL-C:HDL-C, total-C:HDL-C, and apolipoprotein B (p<0.05) compared with pbo. EZE was well tolerated with a safety profile comparable to statin alone.

Table 1. EZE vs. Pbo Added to Statin as % Change from Baseline

Lipid Measure	DM (N=1126)		MS (N=1739)		MD (N=717)	
	Pbo+ statin	EZE +statin	Pbo + statin	EZE + statin	Pbo + statin	EZE + statin
LDL-C	-3.0	-27.8*	-2.2	-25.7*	-2.9	-25.6*
TG	1.2	-11.1*	-2.6	-14.1*	-6.8	-15.9*
HDL-C	-1.3	1.4*	-0.2	2.3*	1.7	3.6*
Non-HDL-C	-3.1	-24.8*	-2.7	-23.4*	-4.3	-23.2*

*p<0.05 (between-treatment difference within subgroup)

“60 – 60 – 60”

ENGINEERING NEGLIGIBLE VASCULAR SENESCENCE FROM A PARTICLE-GRADIENT DRIVEN PERSPECTIVE

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Brown et al. confirmed combination therapy reduces vascular events by 80 to 95% and death from those events by 90%. Resistance to adopt combination therapy, as the standard of vascular care, results in less than optimal reduction (i.e. 25 – 35%) in myocardial infarctions, strokes, angioplasty, vascular stints, coronary artery bypass grafts, and deaths (hereafter referred to as “events”). Larmarck B et al. demonstrated that the number and size of LDL-C particles were significantly associated with the increased odds of IHD. This study attempts to engineer “negligible vascular senescence” in 100 patients by simultaneously modulating multiple risk factors with combination therapy lowered LDL-C Particle numbers from > 1300 nMol/L. to < 1000 nMol/L.

Brown et al.^{1,3} values using:

Tapp Medical Clinic

triple- drug therapy-----simvastatin + niacin ----- Study parameters
 LDL-C – 106 mg/dL-----75 mg/dL-----< 60 mg/dL
 HDL-C – 53 mg/dL-----40 mg/dL-----> 60 mg/dL
 Trig – 134 mg/dL-----126 mg/dL-----< 60 mg/dL

Conclusions: In 100 patients, over a span of three years: 1) LDL-C Particle numbers <1000 nMol/L were achieved 2) No “events” occurred 3) No adverse liver, muscle or renal side effects occurred 4) The Barzilai et al. “Longevity Gene” effects were successfully modeled 5) These outcomes were delivered in a primary care setting.

EFFECTS OF FENOFIBRATE AND EZETIMIBE ON LIPOPROTEIN SUBCLASSES AND LDL SIZE PATTERN IN PATIENTS WITH MIXED HYPERLIPIDEMIA

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In a 12-wk, placebo-controlled trial, coadministered fenofibrate 160 mg/d and ezetimibe 10 mg/d (FENO+EZE) produced complementary, beneficial effects on LDL-C (-20.4%), triglycerides (-44.0%), non-HDL-C (-30.4%), and HDL-C (+19.0%) levels in patients (pts) with mixed hyperlipidemia. This abstract includes effects of FENO and EZE, alone and coadministered, on lipoprotein subclasses. Cholesterol associated with individual lipoprotein subclasses was quantified with the VAP II method and LDL size pattern was determined with S₃GGE. Compared to placebo and EZE alone, treatment with FENO+EZE or FENO produced a redistribution of the LDL particle profile with a decrease in LDL-C4 and an increase in LDL-C2. FENO+EZE resulted in significant reductions in LDL-C1 and 3 beyond those obtained with FENO, consistent with the greater overall LDL-C lowering. FENO+EZE also significantly reduced TG-rich lipoproteins including IDL-C and VLDL-C vs. other treatments. At baseline, >70% of pts exhibited the small, dense LDL pattern B profile. At endpoint, ≥65% of pts in FENO+EZE and FENO groups were shifted to the larger, more buoyant LDL pattern A. Thus, in addition to improving lipids and lipoproteins, FENO+EZE produced favorable effects on atherogenic lipoprotein subclasses and LDL size pattern in pts with mixed hyperlipidemia.

	Placebo (N = 59)	EZE (N = 167)	FENO (N= 171)	FENO + EZE (N = 171)
	(%) ¹	(%) ¹	(%) ¹	(%) ¹
IDL-C	10.0 ^a	-23.8 ^a	-27.0 ^a	-44.0
LDL-C 1	6.3 ^a	-14.0 ^a	-8.0 ^a	-24.9
LDL-C 2	-1.4 ^a	-2.2 ^a	108	80.8
LDL-C 3	1.8 ^a	-6.6	-5.3 ^a	-14.3
LDL-C 4	18.1 ^a	-12.3 ^a	-62.2	-67.3

¹Change = Median percent change; ^ap<0.01 compared FENO + EZE

THE EFFECTS OF THE CURCUMIN ON THE LOWERING-LIPIDS, ANTIOXIDATION AND ANTIATHEROSCLEROSIS

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Curcumin, a compound extracted from *Curcuma Longa*, was found to have the effects of lowering-lipids and antiatherosclerosis as follow. 1. Curcumin reduced the concentrations of the TC, TG, LDL-C and apoB-100 and increased the concentrations of the HDL-C and apoA-I in the animals of dietary hyperlipidemia and the patients suffering from the hyperlipidemia. 2. Curcumin inhibited the oxidation of the LDL with copper-ion in vitro and decreased the concentrations of the lipid peroxides in the serum and liver of the dietary hyperlipidemia animals. It also inhibited oxidizing modification of the macrophages, smooth muscle cell and endothelial cell on the LDL. 3. Curcumin inhibited significantly increasing of fibrinogen and decreasing the activity of PAI-1 and increasing the activity of tPA and the ratio of the tPA to PAI-1 in the hyperlipidemia rabbits. 4. Curcumin increased the expression of the LDL receptor on the bovine smooth muscle cells, B-LCLs and xenopus laevis oocytes. 5. Curcumin increased the level of the LCAT and LPL activity in the plasma of the dietary hyperlipidemia rats. 6. Curcumin could inhibit the proliferation of the smooth muscle cells promoted by 10% serum and ox-LDL. Low concentrations of the curcumin give rise to the apoptosis of the smooth muscle cells.

Except for the lowering-lipids and anti-oxidation the curcumin has even more effects in the anti-tumorigenesis and treatment of the fatty liver. Therefore the curcumin had excellent developing prospect.

TEN YEARS OF PRAVASTATIN (P) PLUS FENOFIBRATE (F) THERAPY IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA (FCH)

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Achieving recommended total and LDL-cholesterol and triglycerides targets could be difficult in patients with FCH. The purpose of this primary prevention study, carried out in our Lipid Clinic, was to evaluate in 46 selected patients resistant to monotherapy (P 40 mg/day or F 200mg/day), the efficacy and safety of association therapy with P (20mg/day) plus F (200 mg/day).

In 36 patients who have reached 10 years of association therapy, compared to monotherapy, P plus F produced a statistically (p<0.01) higher improvement in all lipid and apolipoprotein parameters:

	TC	LDL-C	HDL-C	TG	ApoB	ApoA-1
Baseline(mg/dL)	321	225	38	315	190	110
Monotherapy(%)	-17	-18	+13	-26	-14	+17
P + F (%)	-33	-39	+21	-49	-31	+28

During the 10 years, association therapy was stopped because of gastrointestinal distress (n. 2) or high alanine aminotransferase (n. 3) and for no medical reasons (n. 5). None of the patients suffered from myopathy. Moreover, the cost of the treatment has been decreased.

In conclusion, association therapy with the hydrophilic P and F can be suggested to selected patients with FCH resistant to monotherapy. However, a strict monitoring of liver and kidney parameters and CK activity and patient counselling on the risks and warning signs of myopathy are highly recommended for a rapid diagnosis of myopathy, a possible adverse effect of combination therapy with statins plus fibrates.

CONNECTION BETWEEN ATHEROMATIC CORONARY DISEASE AND LIVER STEATOSIS

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Introduction: The connection between Atheromatic Coronary Disease (ACD) and Liver Steatosis (LS) forms the subject of study in the present paper. To the author's knowledge, there is no reference relating directly ACD and LS, though it is generally acknowledged that there is an indirect relation of these two conditions through their predisposition factors.

Methods: The results of the histological tests of 455 sudden death cases were studied. Our sample contains young and middle-aged adults (41±23 years old), 297 males and 158 females whose tissue preparations were examined in our laboratory during the last five years. Histological cuttings of liver preparations were examined and the percentage of LS was estimated. In addition, histological cuttings of myocardial tissue preparations were examined and estimations of the type of damage of coronary arteries and their stenosis degree were made. Patients with chronic hepatitis, alcoholic or other types of cirrhosis were not taken into account.

Results: Based on the study of the 455 histological tests we conclude that LS is present at all sudden death cases whenever corruption of coronary arteries coexists in the myocardial tissue preparations (p<0.001). The liver steatosis degree was widely distributed from low values (<20%) to high values (90%). The type of damage of coronary arteries was ranged between III and VI concerning cases of stenosis that resulted in obstructions ranged between 15% and 100%. The statistical correlation between the LS degree and the coronary arteries' type of damage and obstruction degree is currently under investigation.

Conclusions: According to our present study there is a potential connection between ACD and LS at people of young or middle age who suffered a sudden death. Hopefully, in the future, the study of the liver's histological structure could be used as an indirect estimation factor of the ACD, an innovation that will significantly contribute to the prevention and opportune prognosis of ACD.

EFFECTS OF ATORVASTATIN ON HDL-APO A-I KINETICS IN DOG.

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Statins are widely used for their positive effects on lipid-lowering and coronary artery disease (CAD), but they are poorly effective in raising HDL-C levels. An emergent therapy to increase HDL-C levels is the use of CETP inhibitors. These molecules could be used in combination with statins to prevent CAD but further studies are needed to evaluate their cumulative effects on HDL metabolism. Dog, a species lacking CETP, treated with a statin could be used to predict the combined effects of these two molecules. Here, we studied the effect of atorvastatin on HDL-apolipoprotein A-I (apoA-I) kinetics in dog, using stable isotopes.

7 beagle dogs were treated with atorvastatin (5mg/kg/day) for 6 weeks. Dogs underwent a primed constant infusion of [5,5,5-²H₃]leucine to determine the kinetics of HDL-apoA-I.

Treatment decreased HDL-apoA-I and HDL-C levels (2.36±0.03 vs 1.55±0.04 g/l, 3.56±0.24 vs 2.64±0.15 mmol/l, respectively, p<0.05 for both). HDL-triglycerides were not affected. HDL-phospholipids were lower (4.28±0.13 vs 3.29±0.13 mmol/l, p<0.05), as well as PLTP activity (0.83±0.05 vs 0.60±0.05 pmol/μl/min, p<0.02). HDL-apoA-I fractional catabolic rate and absolute production rate were higher after treatment (0.006±0.001 vs 0.019±0.004 h⁻¹; 0.592±0.095 vs 1.337±0.254 mg/kg/h, p<0.02 for both).

The present results show that atorvastatin increases HDL-apoA-I production in dog. However, this effect is offset by a higher catabolism that decreases HDL-apo A-I levels. The changes in HDL lipid composition may be involved in the enhanced clearance of HDL-apoA-I. This study provides preliminary informations about the effects of a high dose of atorvastatin on apoA-I metabolism that could occur in humans treated with CETP inhibitor.

NEW INSIGHTS INTO HDL-INDEPENDENT CHOLESTEROL EFFLUX FROM HUMAN MONOCYTE-DERIVED MACROPHAGES

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Cholesterol efflux from human macrophages has been reported to occur in the absence of exogenous lipid acceptors, but is unclear in mechanism. To extend our understanding of this process, the expression of relevant genes, intracellular cholesterol storage and apoE secretion were investigated in human monocyte-derived macrophages using drugs affecting different aspects of cholesterol metabolism. After 24-h incubation in serum-free medium, real-time PCR assays showed that natural (22R-hydroxycholesterol/9-cis-retinoic acid) and synthetic (T0901317 and RO264456) LXR/RXR ligands increased *ABCA1* and *ABCG1* mRNA in macrophages both in the presence and absence of acetylated LDL (acLDL). The ACAT inhibitor avasimibe increased 6-fold *ABCG1* mRNA, whereas no treatment affected *apoE* mRNA. Avasimibe, progesterone and the natural LXR/RXR ligands prevented acLDL-induced cholesterol esterification as measured by HPLC. Cholesterol efflux into acceptor-free medium was enhanced by synthetic LXR/RXR ligands (+76%) and avasimibe (+45%) after acLDL-loading. By contrast, apoE secretion was reduced by drugs affecting cholesterol trafficking but increased by LXR/RXR ligands. Incubation with an anti-apoE antibody virtually removed immunodetectable apoE from the medium, increasing cholesterol storage and decreasing efflux by about 25%. These findings suggest that spontaneous cholesterol efflux from human macrophages (i) was not necessarily promoted by increased cell cholesterol storage, (ii) was increased by compounds that activate *ABCA1* and, to a greater extent, *ABCG1* and (iii) only partially correlated with secretion of apoE, which acted as endogenous cholesterol acceptor.

INFLAMMATION INCREASES HDL BINDING TO PROTEOGLYCAN

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Atherosclerosis is an inflammatory disorder characterized by the retention of lipoproteins by vascular proteoglycans. During inflammation, serum levels of the HDL-containing apolipoprotein, serum amyloid A (SAA), increase dramatically, and levels of its major apolipoprotein A-I (apo A-I) decrease. SAA has proteoglycan binding domains. Its presence in HDL particles might explain the co-localization of apo A-I with proteoglycans in atherosclerotic lesions. To evaluate the effect of inflammation on HDL structure and proteoglycan binding, we studied HDL from C56BL/6 mice injected with lipopolysaccharide (LPS). 24 hours after LPS injection, expression of SAA mRNA increased and apo A-I mRNA decreased in the liver in an LPS dose-dependent manner. Plasma levels of SAA also were dramatically increased by LPS injection (57.3 ± 12.9 μg/ml vs. 13160.0 ± 2241.6 μg/ml, p<0.001). Plasma total cholesterol (81.8 ± 3.9 mg/dl vs. 95.6 ± 2.4 mg/dl, p<0.01) and triglycerides (50.0 ± 4.2 mg/dl vs. 90.6 ± 12.8 mg/dl, p<0.01) increased significantly after LPS injection, but HDL-cholesterol level did not change (63.7 ± 2.3 mg/dl vs. 69.2 ± 2.4 mg/dl, p=ns). FLPC analysis showed that nearly all of the SAA was present in the HDL fractions. MALDI-TOF analysis indicated that inflammatory HDL had high levels of SAA1 and 2 and low levels of apo A-I compared to control HDL. These compositional changes were associated with increased affinity of binding of HDL to the proteoglycan, biglycan, in an in vitro gel shift binding assay (30.7 ± 2.6% vs. 72.7 ± 4.8%, p<0.001). Thus, inflammation changes the composition of HDL in a manner that favors retention of some HDL particles by vascular proteoglycans, which may help explain the co-localization of apo A-I with proteoglycans in atherosclerotic lesions, and which may render inflammatory HDL potentially pro-atherogenic.

ELEVATED HDL DETERMINES RISK OF RECURRENT CORONARY EVENTS WITHIN A HIGH-RISK SUBGROUP OF NON-DIABETIC POSTINFARCTION PATIENTS WITH HYPERCHOLESTEROLEMIA AND INFLAMMATION

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Interaction of inflammation and hyperlipidemia in generating cardiovascular disease risk is not fully understood. We searched for patient subgroups at high risk for recurrent coronary events in 767 non-diabetic postinfarction patients based on the hypothesis that interaction of the risk associated with inflammation with that of atherogenic lipoproteins would further increase risk. We used an exploratory 3-dimensional graphical screening technique as a function of an inflammatory and a lipoprotein-related factor established in previous studies of this population. Results indicated occurrence of a high-risk patient subgroup (N = 149) at high levels of both C-reactive protein (CRP) and total cholesterol, the blood markers best representing the factors, respectively. Kaplan-Meier and Cox multivariate analysis confirmed high risk in the subgroup. Additional risk within the subgroup related to metabolic, inflammatory, and thrombotic blood markers was assessed with the Cox model with results showing only elevated HDL as a significant predictor of risk with hazard ratio, 2.24 (95% CI; 1.12, 4.49; p = 0.023). In addition to high levels of HDL, high-risk patients had larger HDL particles and higher levels of apoA-I. In a study population of non-diabetic postinfarction patients, elevated HDL is predictive, rather than protective, of risk of recurrent coronary events within a subgroup of patients characterized by simultaneous elevations in serum CRP and total cholesterol.

RAISING HDL IIb WITH DIET AND SUPPLEMENTS: ACCOMPLISHING THE IMPOSSIBLE?

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Raising HDL and more importantly, the HDL IIb subfraction, has become the “holy grail” of diet, lifestyle, drug, and supplement interventions, particularly in pts with known/treated coronary artery disease (CAD) who are already taking statins. Based upon previous studies reported by our group, we enrolled 30 consecutive pts, aged 55-85, with documented CAD, in a dietary program that was combined with a regimen of easily obtained supplements from Nationwide stores (Costco, Trader Joe’s) to assess the effect on HDL and HDLIIb particles over a 3-6 month observation period. The “Restore Diet” consisted of a requirement to eat at least one bag of dark green lettuce or “greens”/day, one half cup of mixed raw nuts (walnuts, pistachios, macadamia, pumpkin seeds)/day in two divided doses, avoidance of “white foods,” while incorporating at least a cup of berries/day into meals/desserts. Pts were asked to concentrate on vegetables and fruits.

All pts took 2-4 gms of molecularly distilled fish oil (Omega 3’s, Trader Joe’s), 1500mg of sustained release Niacin in divided doses, 1000 mcg of Folic Acid, Vit C 500mg bid, Mixed Vit E 400mg/day, Magnesium 1000mg/day, Ca Citrate 1000mg/day, CoQ10 50mg/day. All dietary counseling was given by a single physician, who followed the same program. All pts had lipid fractionization at the start and at 3 and 6 month intervals.

Patient acceptance was 100%. HDL levels rose from 40+/-15 to 70+/-10, while percentage of HDLIIb rose from 8+/-16% to 26+/-10% (p<0.001). We conclude that a combination of dietary modifications, with emphasis on “greens” and raw nuts, and easily obtained and tolerated supplements, can produce dramatic elevations in both total HDL and HDL IIb levels in a broad category of pts with CAD.

DIABETIC WOMEN HDL CHOLESTEROL BEHAVIOUR AND EXERCISE TRAINING INDIVIDUALIZATION IN SECONDARY PREVENTION OF CORONARY ARTERY DISEASE

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Objectives: HDL-cholesterol above 40mg/dl is a risk category. Our study analyses the effect of cardiovascular rehabilitation on HDL behavior and on other lipidic components depending on gender and on the baseline levels of the lipids in diabetics. **Method:** We followed 393 diabetics included in a cardiovascular rehabilitation program. We created the HDL categories in order to stratify the patients at baseline in four groups: men with elevated/low HDL; women with elevated/low HDL. **Results:** Diabetic women had a significant increase of HDL-cholesterol, both in the group with baseline HDL < 40mg/dl (p=0.01) and in the group with baseline HDL ≥ 40mg/dl (p=0.04), comparative to men. HDL had a better outcome in the population with low baseline HDL (p=0.004). Exercise training leads to a significant increase of effort capacity in women with baseline HDL < 40mg/dl than in those with baseline HDL ≥ 40mg/dl (p=0.04). LDL decrease was significant in women with HDL < 40mg/dl (p< 0.0001). TC and TG changes depending on HDL and gender were not significant. HDL multiple regression equation showed a significant correlation for gender (p=0.03) and for ΔTC (p=0.05); correlation with effort capacity was significant in men with low baseline HDL and women with high baseline HDL (p=0.05). Other correlations were not significant. **Conclusions:** HDL-cholesterol increases more in diabetic women than in men after the cardiovascular rehabilitation program. Exercise training only is beneficial in diabetic women with baseline HDL ≥ 40mg/dl, meaning that there are other factors that lead to HDL improvement. In clinical practice we have to consider the impact of gender while assessing individual cardiovascular risk and also while considering therapeutic options.

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

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Objective: Scavenger receptor class B type I (SR-BI) is a major receptor for HDL in the liver, which is the terminal of reverse cholesterol transport. 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 aim of the present study was to know the effect of probucol on the expression of SR-BI and its underlying mechanism. **Methods and Results:** We found that probucol increased the expression of SR-BI proteins in *in vitro* human liver cells and *in vivo* rabbit model. However, this effect was not observed in wild type C57Bl6 mice. 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. In order to know the underlying mechanism for the observed species-specific effect, we conducted the following host-swap experiments, in which SR-BI was transfected or expressed in heterologous cells or hosts. Probucol did not increase human SR-BI protein in the liver of transgenic mice carrying the entire human SR-BI genome. Probucol could stabilize even murine SR-BI, when transfected into a human cell line, HepG2, whereas human SR-BI was not stabilized in a mouse hepatoma cell line, Hepa 1-6, treated with probucol. **Conclusions:** Probucol increases hepatic SR-BI protein possibly through species-specific stabilization of the protein.

OXIDISED HDL SUBFRACTIONS ALTERS THE RELEASE OF PRO-INFLAMMATORY CYTOKINES FROM HUVECS

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Introduction: High density lipoprotein (HDL) exhibits a range of anti-atherogenic effects with the most widely accepted role being its involvement in reverse cholesterol transport. However, HDL has also been shown to exert additional anti-atherogenic effects on vascular cells.

Aims: To assess if oxidation of HDL subfractions, isolated by a rapid methodology, alters their anti-atherogenic potential.

Methods: Crude HDL was isolated from fasting EDTA-plasma by a rapid density sedimentation method. HDL was then fractionated into HDL₂ and HDL₃ by two sequential ultracentrifugation steps, each requiring 2 hours ultracentrifugation at 541 000 g_{max}. HDL subfractions were characterised by single radial immunodiffusion for apo A-I, apo A-II, apo B, apo C-II and transferrin. HDLs were subjected to Cu²⁺-mediated oxidation using CuCl₂, with the *in vitro* effects of HDL oxidation being examined in human umbilical vascular endothelial cells (HUVECs). The release of pro-inflammatory markers into the cell culture supernatant was assessed using commercially available ELISA kits for 6-keto-PGF_{1α} (PGI₂), TNF-α, IL-6 and sVCAM-1, sICAM-1.

Results: Mean percent (±SD) recovery of HDL₂ + HDL₃ apoproteins from crude HDL were apo A-I 101.2±18.5; apo A-II 97.1±16.8; apo C-II 100.7±21.0. HDL subfractions were devoid of apo B contamination. We also found transferrin enrichment of the larger HDL₃ subfraction [5.6 vs. 13.3 μg mL⁻¹ (P = 0.001)]. Examination of cell supernatant following exposure to oxidised HDL subfractions demonstrated decreased levels (non-oxidised vs. oxidised) of 6-keto-PGF_{1α} (HDL₂ 65.5 vs. 37.6; HDL₃ 71.1 vs. 60.5 pg mL⁻¹), increased TNF-α (HDL₂ 8.3 vs. 10.64; HDL₃ 20.8 vs. 49.2 pg mL⁻¹) and IL-6 (HDL₂ 18.7 vs. 26.7; HDL₃ 10.1 vs. 34.7 pg mL⁻¹). Levels of sICAM-1 and sVCAM-1 remained unaltered.

Conclusions: We have reported a rapid means of isolating HDL₂ and HDL₃, and shown that oxidation of these subfractions modulates *in vitro* expression of pro-inflammatory cytokines.

IMPORTANCE OF HDL-C ON CARDIOVASCULAR PREVENTION. RECOMMENDATIONS OF HDL-FORUM IN SPAIN

Millan, J., Ascaso J, Fernandez-Cruz, A, Gonzalez Santos P, Hernandez Mijares A, Mangas A, Pallardo LF, Pedro-Botet JC, Perez Jimenez F, Pia G, Pintó X, Plaza I, Rubiés J, Querol M, Ros C, on behalf of HDL-Forum of Spain

The management of high serum LDL-c is only a part of the problem represented by dyslipidemia as a cardiovascular risk factor. Coronary Heart Disease (CHD) occur in individuals with normal LDL-c even in treatment with HMGCoA-reductase inhibitors. This feature is related with other lipid factors particularly low serum HDL- levels.

Under the auspices of Spanish Society of Atherosclerosis and the Spanish Society of Diabetes, a number of members (HDL-Forum) has recently reported specific recommendations for intervention according with different conditions: Patients with CHD and/or equivalent (10 year risk > 20%), low serum HDL-c and 10-year overall risk < 20 %, and secondary dyslipemias: renal disorders, HIV infection, hypertension and antihypertensive drugs

HDL-Forum recommendations emphasize on several "Low HDL-c conditions" (< 40 mg/dl in men; < 50 mg/dl in women and/or total-c/HDL-c ratio > 5 in men or > 4,5 in women) that must be considered as risk categories: hypertriglyceridemia, type 2 diabetes mellitus, metabolic syndrome and cardiovascular risk > 20 % in primary prevention; and CHD or equivalents in secondary prevention.

Lifestyle changes and drug therapy will be considered. In general, fibric acid derivative therapy is indicated when low HDL-c occurs in association with: CHD or extracoronary atherosclerotic disease, high cardiovascular risk, hypertriglyceridemia, metabolic syndrome, or diabetes mellitus. The treatment with a combination of a statin and a fibric acid derivative will depend on the baseline serum LDL-c levels.

HDL-CHOLESTEROL IS HIGHER IN WOMEN THAN IN MEN WITH ACUTE CORONARY SYNDROME WITHOUT ST-ELEVATION

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Background. Increase in HDL-cholesterol is associated with a significant reduction in acute coronary events. HDL-cholesterol is higher in women than in men throughout life. It is less well known if this is the case in acute coronary syndrome (ACS). Our aim was to compare lipids between men and women with ACS without ST-elevation.

Methods. In 44 women and 87 men with ACS without ST-elevation on ECG percutaneous coronary interventions followed medical therapy in case of recurrent chest pain and/or rhythmic and/or hemodynamic instability. Baseline characteristics, admission lipid profile and reinfarctions at 6 months were registered.

Results. Between men and women we observed significant differences in age (59.4±10.7 vs 66.2±9.6, $p < 0.05$), arterial hypertension (51.7% vs 77%, $p < 0.05$), smoking (33.3% vs 11.4%, $p < 0.05$), physical activity (91.9% vs 77.2%, $p < 0.05$) and mean HDL-cholesterol level (1±0.3 mmol/L vs 1.2±0.3 mmol/L, $p < 0.05$) but nonsignificant difference in total cholesterol, triglyceride, LDL-cholesterol levels and reinfarction rate. HDL-cholesterol < 1 mmol/L was less likely in women than in men (30% vs 52.9%, $p < 0.05$, OR 0.37, 95% CI 0,17 to 0.80).

Conclusions. Higher HDL-cholesterol in women than in men could be among factors for later occurrence of ACS without ST-elevation in spite of more frequent comorbidities in women than in men.

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