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

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The cardioprotective effects of omega-3 fatty acids (ω-3 FA) have been documented in both epidemiological studies and randomized controlled trials. They are presumed to reduce susceptibility to fatal arrhythmias. This could be mediated by changes in cardiac membrane lipid composition or extra-cardiac factors (e.g., autonomic balance). Omega-3 FA may also have antiinflammatory properties. The effects of AHA-recommended intakes of ω-3 FA on cardiac function, autonomic balance and inflammatory cytokines 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 in a cross-over design. At the end of each period, a non-invasive cardiovascular profile was obtained (CVProfilor DO-2020, Hypertension Diagnostics) which included measures of large and small artery 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 (67±11 to 63±12 bpm, p=0.01), and increased left ventricular ejection time (319±27 to 329±29 ms, p=0.03) and stroke volume (80±14 to 85±15 mL, p=0.001). HR recovery at 1-minute post exercise tended to increase (n=14; -25.8±10.2 to -29.1±10.2 bpm, p=0.07). 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 lower serum levels of inflammatory cytokines, they did produce a favorable shift in cardiac autonomic balance. This would be expected to reduce risk for sudden cardiac death regardless of changes in myocardial lipid membrane FA composition.

## THE ANTI-ATHEROGENIC EFFECT OF ALLICIN: POSSIBLE MODE OF ACTION

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***Background:*** Garlic (*Allium sativum*) has been suggested to affect several cardiovascular risk factors. Its anti-atherosclerotic properties are attributed mainly to allicin that is produced upon crushing of the garlic clove. Previous studies used various garlic preparations in which allicin levels were not well defined.
***Objective***: In the present study we evaluated the effects of pure allicin on atherogenesis in experimental mouse models.
***Methods and Results:*** Daily dietary supplement of allicin, 9 mg/kg body weight, reduced the atherosclerotic plaque area by 68.9%, 56.8% and 65.6% in apoE -/-, LDL-R -/- and C57Bl/6 mice respectively, as compared to control mice. Low density lipoprotein isolated from allicin-treated groups was more resistant to $CuSO_4$-induced oxidation *ex vivo* than LDL isolated from control mice. Incubation of mouse plasma with $^3$H-labeled allicin showed binding of allicin to lipoproteins. By using ESR we demonstrated reduced $Cu^{2+}$ binding to LDL following allicin treatment. LDL treatment with allicin significantly inhibited both native-LDL and oxidized-LDL degradation by isolated mouse macrophages.
***Conclusions***: By using a pure allicin preparation, we were able to show that allicin may affect atherosclerosis not only by acting as an antioxidant, but also by other mechanisms such as lipoprotein modification and inhibition of LDL uptake and degradation by macrophages.

## IMPROVEMENT OF FIBRATE ACTION BY A COMBINATION TREATMENT OF THE DRUG PLUS 9-CIS β-CAROTENE-RICH POWDER OF THE ALGA *DUNALIELLA BARDAWIL*

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PPARs function as ligand-dependent transcription factors, which upon activation heterodimerize with the 9-cis retinoic acid receptor (RXR).
***Hypothesis:*** Since 9-cis β-carotene isomer is a natural precursor of 9-cis retinoic acid, the ligand of RXR, we assayed the hypothesis that a dual treatment with fibrate plus 9-cis β-carotene-rich-powder of the alga *Dunaliella bardawil* would improve the therapeutic effect of the drug on HDL-cholesterol levels.
***Methods:*** Twenty men, taking fibrates for at least 6 weeks were recruited to the study. Their plasma HDL cholesterol levels were below 40mg\dl and their plasma triglycerides levels were over 200mg/dl.
***Results:*** Fibrate plus 9-cis β-carotene treatment increased plasma HDL cholesterol by 24.5% (p=0.002) comparing to fibrate alone (36.5±9.9 mg per deciliter vs. 29.3 ±6.8 mg per deciliter, respectively). The dual treatment increased HDL-cholesterol levels in transgenic human apoAI mice and reduced atherosclerotic lesion area in both LDLR-/- and apoE-deficient mice.
***Conclusions:*** The results of these experiments suggest that a combination treatment of fibrate plus 9-cis β-carotene amplifies the effect of the drug on HDL-cholesterol levels.

## PLANT STEROLS AND STANOLS DO NOT AFFECT ANTIOXIDANT STATUS OR OXIDATIVE STRESS IN PATIENTS ON STABLE STATIN TREATMENT

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Plant sterol or stanol ester consumption lowers serum LDL cholesterol. Unfortunately, it has also been suggested that these compounds may diminish antioxidant status, which might increase oxidative stress, an important factor in atherosclerosis. We therefore hypothesized that consumption of plant sterols and stanols would not only affect fat-soluble antioxidant levels and possibly enzymatic antioxidant systems, but also parameters of oxidative stress.
To test this, 45 patients on stable statin treatment consumed margarines with no added plant sterols or stanols for 4 weeks and were then divided into 3 groups of 15 subjects. For the next 16 weeks, the first group continued with the control margarine, and the other two groups with either a plant sterol or stanol ester enriched margarine (2.5 g plant sterols or stanols as its fatty acid esters / day). LDL cholesterol decreased by 2.0 ± 3.9% (mean ± SEM) in the control group, by 7.5 ± 3.2% in the plant sterol group and by 12.1 ± 3.8% in the plant stanol group. Reductions in ApoB100 were comparable to those of LDL. Fat-soluble antioxidant concentrations (tocopherols, carotenoids) were decreased, though not significantly. Also enzymatic antioxidant systems (superoxide dismutase, glutathione peroxidase) were not affected. Oxidized LDL concentrations (standardized for apoB100) were non-significantly lowered (p=0.918) by respectively 4.4% and 1.8% in the plant sterol and stanol group, as compared to the control group. Also changes in plasma malondialdehyde (MDA) levels were not significantly different between the three groups. In conclusion, in patients on stable statin treatment, 16 weeks plant sterol or stanol ester consumption does not affect antioxidant status or parameters of oxidative stress.

## EFFECTS OF MODERATE CONSUMPTION OF SICILIAN RED WINES ON SOME CARDIOVASCULAR RISK FACTORS

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Aim of the study is to evaluate whether Sicilian red wine consumption is associated with lower cardiovascular risk. 48 subjects of both sexes (aged between 35- 65 years) nondrinkers or rarely drinkers of moderate red wine intake were selected. All subjects were divided into two groups (Group A and Group B), assigned to receive with a cross-over design 250 ml/die (during the meals) of one of 2 types of Sicilian red wines (Nero d'Avola and Etna Torrepalino respectively). At all visits (-14 days, basal, +4 and +8 weeks) the following parameters were measured: Blood glucose, total cholesterol and triglycerides (by enzyme kit methods), HDL-C (by selective precipitation with dextran-magnesium chloride), LDL-C (by calculation with the Friedewald formula), LDL/HDL ratio, APO A1 and B (by radial immunodiffusion), Lp(a) (ELISA), plasma C-reactive protein (High Sensitivity), TGFbeta1 (ELISA), D-Dimer (Turbiquant), Factor VII (Coagulant), PAI Antigen (ELISA), t-PA Antigen (ELISA), Fibrinogen (Coagulant), oxidized LDL antibody (ELISA), Total plasma antioxidant capacity (FRAP method). At the end of red wine intake period, HDL-C was significantly ($p<0.01$) increased and the LDL/HDL ratio was significantly ($p<0.05$) decreased in Group A and in Group B, while APO A1 was significantly ($p<0.05$) increased only in Group A. At the end of red wine intake period, in Group A and in Group B Fibrinogen ($p< 0.01$ and $p< 0.005$ respectively), Factor VII ($p< 0.01$ and $p<0.05$ respectively), plasma C-reactive protein ($p< 0.005$ and $p<0.05$ respectively) and oxidized LDL antibody ($p< 0.05$) were significantly decreased, while TGFbeta1 ($p<0.05$), t-PA ($p<0.005$), PAI ($p<0.005$) and total plasma antioxidant capacity ($p< 0.005$) were significantly increased. Our results show a positive effect of these Sicilian red wines on many risk factors, suggesting a moderate consumption of red wine in the adult population as component of Mediterranean diet.

## PLANT STANOLS LOWER LDL CHOLESTEROL WITHOUT IMPROVING ENDOTHELIAL FUNCTION IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Children with familial hypercholesterolaemia (FH) are already characterized by impaired endothelial function, a hallmark of early atherogenesis. This emphasizes the need for early treatment of children with FH. Currently, options for treating high cholesterol levels in children with FH are limited. Therapy with HMG-CoA reductase inhibitors (statins) has been shown to restore endothelial function in FH children, but most statins have not been registered for use in children. Plant sterols or stanols also lower cholesterol levels by inhibiting cholesterol absorption in the small intestine. In the present study we examined the effect of stanols on lipids and endothelial function in young FH children.
We included 41 FH children, aged 7-11 years, in a double-blind crossover trial consuming 500 ml of a low-fat yogurt enriched with 2.0 g of plant stanols (mainly sitostanol) and a low-fat placebo yogurt for 4 weeks, separated by a 6-week wash-out period. Lipid profile and endothelial function were assessed after both treatment periods. Endothelial function was measured as flow-mediated dilation (FMD) of the brachial artery by means of ultrasonography and a wall tracking system.
A daily intake of 2.0 g stanols decreased total cholesterol by 7.6% and LDL cholesterol by 9.1% in children with FH as compared to placebo. However, this reduction of LDL cholesterol did not improve FMD, which was 10.5 ± 5.1% after stanol and 10.6 ± 5.0% after placebo consumption ($p= 0.852$).
In conclusion, the present study confirms that stanol consumption reduces LDL cholesterol in children with FH. However, stanols do not improve the endothelial function in children with FH, which might be due to a threshold of LDL-C lowering before endothelial function can improve.

## DIETARY CHANGES IN FH-CHILDREN PARICIPATING IN A CONTROLLED CLINICAL STUDY

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***Introduction*** Individuals heterozygous for familial hypercholesterolemia (FH) have an increased risk to develop atherosclerosis and coronary artery diseases if compared to individuals without FH. Therefore, in treatment guidelines of high risk groups, restriction of saturated fat intake and cholesterol in conjunction with LDL cholesterol lowering drugs is advised. ***Objective*** In this study we investigated the effect of frequent dietary instructions on the diet of FH children. ***Methods*** This study was part of a trial in which the safety and cholesterol lowering effect of a statin was studied in FH children. Fifty children (aged 10 - 17 years) were asked to keep a food diary for three days before the start of the trial (t=0). Once included, they received dietary instructions from the trial nurse every time the clinic was visited. After 1 year (t=1) the children were asked to keep a food diary again. Food intake at t=1 was compared with that of t=0 as well as to the Recommended Dietary Allowance (RDA) and Dutch National Food Consumption Survey standards. ***Results*** Intake of subjects with FH met the RDA fat standards. After one year of dietary recommendations under trial conditions, intake of fat and saturated fat was reduced with 3 and 2%. Intake of carbohydrates, especially the mono- and disacharides, increased 3%. Intake of proteins and kilocalories was 14% and 1698 kcal, respectively 14% and 1760 kilocalories and remained unchanged if compared to t=0. There was no significant difference mean carbohydrates intake, but subjects with FH consumed less saturated fat than subjects of age and sex matched controls. ***Conclusions*** The mean intake of fat and saturated fat was reduced after 1 year of dietary recommendations every visit to the clinic. The amount of carbohydrates, especially of mono- and disaccharides, were elevated, which suggests that fatty products were replaced by sweet products. The findings indicate that diet instructions, parallel to the favourable effect of lipid modifying drugs, contributes to the creation of a more favourable disease risk profile in subjects prone to cardiovascular disease.

## L-CARNITINE TREATMENT IN HEMODIALYSIS PATIENTS

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The correction of impaired lipid metabolism can help to reduce the complications related to atherosclerosis in hemodialysis (HD) patients. Anemia that is also common in these patients could cause multi organ dysfunction.The aim of this study was to examine the influence of L-carnitine therapy and erythropoietin on dislipidemia and anemia in HD patients. A number of 78 HD patients (43 men and 35 women), mean age 51years were divided in 2 groups (for lipid examination): with and without L-carnitine therapy (1 gr. i.v.) after every HD session; in 4 groups (concerning anemia): Igr. – under L-carnitine therapy, IIgr. - under erythropoietin therapy, IIIgr. - under L-carnitine and erythropoietin therapy and IVgr. - as a control one (without any therapy). Blood count was determined by Hematological Analyser, Cobas Micros-OT 8 and tryglycerids and cholesterol were determined by Biochemical Analyser, dry chemistry Vitros 250, before and after the 6 month therapy. The results showed decreased tryglyceridemia in patients on L-carnitine therapy, n=34, from 2.6 ± 1 mmol/l before to 2.1 ± 1.1 mmol/l after the therapy ($p<0.05$), as well as for cholesterol level, 5.08 ± 1.4 before and 4.33 ± 1.2 mmol/l after the therapy. Hemoglobine was found to be elevated in the II (n=28) and III (n=18) group, from 88.7 ± 21 to 101.8 ± 15 g/l ($p<0.01$) for the former and from 90.2 ± 13 to 103 ± 18 g/l ($p<0.05$) for the latter. Red blood cell count increased from 2.87 ± 0.8 to 3.39 ± 0.6 x $10^{12}$/l in II gr. ($p<0.01$) and from 2.75 ± 0.6 to 3.24 ± 0.6 x $10^{12}$/l in III gr. ($p<0.03$). No significant differences in hemoglobine and red blood cells were found in I group.
Due to obtained results in this study, L-carnitine treatment showed hypertriglyceridemia improvement and erythropoietin showed his beneficial influence on anemia in HD patients.

**A ONE-YEAR STUDY OF THE EFFECT OF LACTO-TRIPEPTIDES IN A SOUR MILK ON BLOOD PRESSURE OF HYPERTENSIVE SUBJECTS**

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Calpis sour milk is a cultured milk prepared by fermenting skim milk with *Lactobacillus helveticus*, containing two lactotripeptides, Val-Pro-Pro and Ile-Pro-Pro as fermentation products. These two principles are demonstrated to have an ACE inhibitory effect to lower blood pressure (BP) of SHR, and also on hypertension of human subjects by a double blind study for 8 weeks. In this study we observed the effect on BP of 26 hypertensive subjects for 52 weeks. At the start we advised a total of 39 hypertensive patients who were on hypotensive drugs, but have an occasional elevation in BP above 150/90mmHg levels to take 100ml of Calpis sour milk (containing VPP 1.5mg and IPP 1.1mg) daily in addition to prescribed drugs. However, 13 subjects could not continue to take and dropped out of the study. This group was served as the control. The patients were asked to visit clinic once a month, when checked compliance for both medication and drink, body weight, BP and serum lipids.. The starting BP averaged as 150+-10/83+-9mmHg in sour milk(SM) group (n=26, 70+-12yo) and 145+-10/81+-6mmHg in control group (n=13, 71+-4yo). After 4 weeks BP dropped by 14+-4nnHg in SM group and 8+-3mmHg in control group. After 8weeks BP of the SM group remained constantly lower than that of the control group, the difference between the two groups were statistically significant at 12, 16, 28 and 52 weeks. Serum LDL-c, triglycerides, and fasting blood glucose did not change in both groups, while HDL-c in SM group tended to elevate in 52 weeks.

**BENEFICIAL EFFECT OF LOW CARBOHYDRATE IN LOW CALORIE DIETS ON VISCERAL FAT REDUCTION IN TYPE 2 DIABETIC PATIENTS WITH OBESITY**

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The adequate composition of carbohydrate and fat in low calorie diets for type 2 diabetes mellitus patients with obesity is not fully established. The aim of this study was to investigate the effects of low carbohydrate diet on glucose and lipid metabolism, especially on visceral fat accumulation, and comparing that of a high carbohydrate diet. Obese subjects with type 2 diabetes mellitus were randomly assigned to take a low calorie and low carbohydrate diet (n=11, 1000 kcal/day, protein: carbohydrate: fat = 25: 40: 35) or a low calorie and high carbohydrate diet (n=11, 1000 kcal/day, protein: carbohydrate: fat = 25: 65: 10) for 4 weeks. Similar decreases in body weight and serum glucose levels were observed in both groups. Fasting serum insulin levels were reduced in the low carbohydrate diet group compared to the high carbohydrate diet group (-30% versus -10%, $p<0.05$). Total serum cholesterol and triglyceride levels decreased in both groups, but were not significantly different from each other. High-density lipoprotein- cholesterol (HDL-C) increased in the low carbohydrate diet group but not in the high carbohydrate diet group (+15% versus 0 %, $p<0.01$). There was a larger decrease in visceral fat area measured by computed tomography in the low carbohydrate diet group compared to the high carbohydrate diet group (-40 $cm^2$ versus –10 $cm^2$, $p<0.05$). The ratio of visceral fat area to subcutaneous fat area did not change in the high carbohydrate diet group (from 0.70 to 0.68), but it decreased significantly in the low carbohydrate diet group (from 0.69 to 0.47, $p<0.05$). These results suggest that, when restrict diet was made isocaloric, a low calorie/low carbohydrate diet might be more effective treatment for a reduction of visceral fat, improved insulin sensitivity and increased in HDL-C levels than low calorie/high carbohydrate diet in obese subjects with type 2 diabetes mellitus.

**SELECTED RISK FACTORS OF ATHEROSCLEROSIS AND BREAST FEEDING**

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We enrolled 47 mother + infant pairs (before the labour) that were followed until the infant's 6th postnatal month. We determined total (TC) and HDL cholesterol (HDL), triglycerides (TG), apolipoprotein AI (apoA) and B (apoB), oxidized LDL (oxLDL), antibodies against $Cu^{2+}$-oxidized LDL (oxLDLAb), thiobarbituric acid reacting substances (TBARS) and paraoxonase (PON1). LDL cholesterol (LDL) was calculated according to Planell.
In the 3rd month showed fully breast fed infants (vs. formula fed infants) higher levels of TC ($p<0.001$), apoB ($p<0.001$), LDL ($p<0.01$), oxLDL ($p<0.005$), TBARS ($p<0.001$) and lower titres of oxLDLAb ($p<0.001$). Differences in oxLDL and TBARS in the 3rd month weren't statistically significant after a correction for LDL (oxLDL/LDL and TBARS/LDL). Higher oxLDL and TBARS in breast fed infants are probably caused by better supply of LDL than by enhanced oxidative stress. Breast fed infants are characterized by a low immune response to oxidatively modified LDL.

**EFFECT OF CONJUGATED LINOLEIC ACID (CLA) IN MILK FAT ON BLOOD LIPIDS, C-REACTIVE PROTEIN, OXIDATIVE STRESS AND ARTERIAL ELASTICITY IN YOUNG MEN**

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Conjugated linoleic acid (CLA) has in animal studies studies been shown to have chemoprotective properties. Our aim was to examine the effect of a commercial CLA mixture (containing 39.4% cis 9 trans 11 and 38.5% trans 10 cis12) on blood lipids, insulin, glucose, the inflammatory biomarker C-reactive protein (CRP), in-vivo lipid peroxidation, and arterial elasticity in young healthy men. We compared the effect of a diet high in milk fat supplemented with 4.5 grams of CLA with a control milk fat with a very low content of CLA (difference in CLA content was 3 grams) in a study with double-blind, randomized, parallel design. 61 healthy young men substituted 115 g of their daily fat intake by a test butter during an intervention period of 5 weeks. Blood and urinary samples were obtained and arterial elasticity measured before and after each intervention period. Our results showed that the two CLA isomers were incorporated in measurable amounts in plasma cholesterol esters in 15 participants (out of 19) and in plasma phospholipids in 13 participants (out of 19). Compared to the control diet the CLA mixture increased urinary 8-iso-PGFα 95 % ($P=0.003$) and tended to increase plasma insulin ($p=0.07$) and arterial elasticity ($p=0.08$). The CLA mixture did not affect plasma CRP, glucose, blood lipids and lipoproteins. We conclude that a CLA mixture (cis 9 trans 11 and trans 10 cis12) given as supplementation increases oxidative stress and may affect insulin sensitivity unfavourably by increasing plasma insulin without affecting plasma glucose. In contrary, the CLA mixture tend to affect arterial elasticity measured by a volume-oscillometric method in a beneficial way.

## THE EFFECT OF n-3 FATTY ACIDS ON SOLUBLE ADHESION MOLECULES IN PATIENTS WITH RENAL FAILURE

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INTRODUCTION Adhesion molecules are expressed on the surface of the endothelium as a part of an inflammatory response. Plasma levels of soluble adhesion molecules, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and p-selectin are believed to reflect this process. Patients with chronic renal failure (CRF) are at high risk of cardiovascular disease and several studies have shown increased levels of soluble adhesion molecules in patients with CRF. n-3 polyunsaturated fatty acids (PUFA) have protective cardiovascular effects but there are diverging results regarding the effect of n-3 PUFA on soluble adhesion molecule levels. The aim of this study was to examine the effect of n-3 PUFA on levels of soluble adhesion molecules in patients CRF.
MATERIAL AND METHODS Fifty-eight patients with CRF, defined as serum creatinine >150 µmol/l and < 400 µmol/l, were included. Patients were randomised to treatment with 2.4 g n-3 PUFA or placebo (olive oil) for eight weeks. Fasting blood samples were obtained before and after treatment. Compliance was assured by measuring levels of n-3 PUFA in granulocytes.
RESULTS At baseline, there were no correlations between levels of n-3 PUFA and VCAM, ICAM or p-selectin. Compared to the control group there were no effect on ICAM or VCAM levels after treatment with n-3 PUFA. There was no effect of n-3 PUFA on p-selectin in men (94±32 ng/ml vs 96±40 ng/ml), but a significant effect was seen on p-selectin levels in women (93±39 ng/ml vs 88±35 ng/ml, p<0.05).
CONCLUSION In patients with CRF there were no effect on levels of ICAM or VCAM after treatment with n-3 PUFA for eight weeks. However, in women, a significant beneficial decrease was seen in the level of p-selectin.

## CARDIO_RESPIRATORY FITNESS.BODY FATNESS,CORONARY HEART DISEASE RISK FACTORS IN ADOLESENCE MALE

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The purpose of the persent study is to examine the relationship of association between coronary fitness in 15 to 18 yr-old adolescence boys.On hundred sixty healthy young males (aged 16.25+_1.12 years; height 168.32+_8.26 cm; and body mass8.32+_12.5kg;means+_SD)were selected from among 15000 high school students.The clustering method was used for sampling (two high school were selected from each region;and 40 students from each high school and 10 subjects for age).Subjects completed an informed conset form,and health history questionnaire.Measurements included the percent age of body fat (PBF),anthropometry,aerobic power(VO2max),and CVD risk(blood pressure),total cholesterol,low-density lipoprotein(LDL),high-density lipopotein(HDL),and triglycerides.Serum lipid measurement was only performed on 36 subjects (6 from each age range).Pierson correlation cofficient was performed data,and the minimal level of significance for this study was set at P<_0.05 There was a significant relationship between the aerobic fitness and level of TG(P<_0.05).In assessment of relationship between the body composition indices and serum lipids, there was a significant relationship between body mass and TG and HDL; between %BF and TG;and TC;and between BMI and TG(P<_0.05).There was also significant relationship between w and BMI with SBP and DBP,between PBF and DBP(P0.05). There results indicate that body compositions were related to CHD risk factors (serum lipids and blood pressure).However,no significant or weak relationship existed between aerobic fitness and CHD risk factors.The results of this study showed that in youth,health status in this age was more affected by body fat,in spite of the obesity during childhood and adolescence is considered as a major preventive factor for CHD.

## 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 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. Although the effects of exercise (i.e. the combination of concentric and eccentric muscle contraction) on metabolic parameters have been extensively investigated, there are no data on the specific metabolic effects of concentric versus those of eccentric muscle work in humans. We hypothesized that both concentric and eccentric muscle exercise affect lipid and glucose metabolism.
Methods: Forty-five healthy sedentary non-diabetic 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. The exercise comprised a steady upward/downward hike over a difference in altitude of 510 meters. For the way back, a cable car was used. Compliance to the exercise regimen was measured by personal records of participants and by electronic records from the cable car tickets. 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 triglyceride curve was significantly lowered only along with concentric exercise (by 11.0%; p = 0.037). LDL cholesterol was reduced significantly both along with concentric (by 10.2%; p <0.001) and eccentric exercise (8.9%; p = 0.001). The area under the glucose curve was improved by 4.5% (p = 0.145) along with concentric exercise, and by 8.2% (p = 0.027) along with eccentric muscle exercise.
Conclusions: We conclude that both concentric and eccentric muscle training have favourable effects on both lipid and glucose metabolism. In healthy sedentary individuals eccentric muscle training improves glucose tolerance more than concentric muscle training. 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.

## SERUM LIPIDS AND LIPOPROTEINS LEVELS IN GREECO_ROMAN WRESTLERS

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Research has shown those regular physical activities and endurance training reduce the risk of cardiovascular disease due to the decreasing harmful substance within the body.The purpose of this research was to assess the level of serum lipids and lipoprotein of Greco Roman Wrestlers.20 young male men aged 18-24 voluntarily participated in this research.They were randomly assigned into two groups participating in Greco Roman Wrestlers training 3 to4 sessions per week.Blood samples were collected and analyzed.Biood components such as HDH,LDL,TC were measured.The results of analysis showed that there was no significant difference between this parameter following participation in the exercise programs.Complete details of procedures and assessments were explored and discussed.

Key words:lipids,lipoprotein,Greco RomanWrestlers

**CHOLESTEROL, TRIGLICERIDE AND GLICEMIA IN HYPERTENSIVE WASH WORKERS**

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Risk factor assessment in different professions has important part in cardiovascular disease prevention.
Purpose: The aim of our study was to evaluate risk factors: cholesterol, trigliceride and glicemia in hypertensive wash workers in Belgrade.
Design and methods: We analyzed data from regular check ups examinations in wash workers in 2003. There were 252 male workers, average age 41.5 years. Hypertensive workers were those with blood pressure 140/90 mmHg or higher or use hypertensive therapy. There were 71 hypertensive and 181 normotensive workers. Cholesterol, trigliceride and glicemia analyzed after 12 hours without eating.
Results: 1. Mean values in our group were: cholesterol 6.45 mmol/l, trigliceride 2.34 and glicemia 5.6; 2. Mean values in normotensive group were: cholesterol 6.38 mmol/l, trigliceride 2.25 and glicemia 5.55; 3. Mean values in hypertensive group were: cholesterol 6.61 mmol/l, trigliceride 2.54 and glicemia 5.72. Mean values for cholesterol, trigliceride and glicemia were higher in hypertensive group.
Conclusion: 1. Mean values in cholesterol, trigliceride and glucemia were above normal according to European recommendations in whole group. 2. These values were higher in hypertensive group. 3. Our results suggest the need off better risk factor control.

**HYPERTENSION CONTROL AND METABOLIC SYNDROME IN A SAMPLE OF "BLUE-COLLAR" EMPLOYEES IN SLOVAKIA.**

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Introduction: Slovakia belongs to the countries with the highest cardiovascular standard death rates in Europe (up to 990 per 100 000). Better hypertension control may significantly improve the cardiovascular mortality.
Aim of the study: To evaluate in the pilot study hypertension control according the recent guidelines in relationship to the classic risk factors.
Patients and methods: Sample of "blue-collar" employee population was followed-up. Standardized questionnaire and blood pressure measuring instrument (BP TRU TN, fulfilling the standards of AAMI and BHS), thus eliminating inter and intra observer errors were used.
1189 (750 men and 448 women) of the fertilizer production factory in a rural population area of Slovakia have been evaluated with response rate up to 95%. 236 were in the group aged 40-49 yrs, 217 aged 50-59, 168 aged 30-39 and 115 were younger than 30 years.
Results: According the recent guidelines, 24% of patients with hypertension were found to be controlled, 18% only partially controlled and 59% of the investigated patients were uncontrolled; 158 were unaware of the disease. 30% of the examined subjects were smokers. BMI index in men was shown to increase with age from 26, through 28 up to 29 in the followed groups, waist circumference from 94, through 98 up to 99 cm in the followed groups respectively.
Conclusions: High prevalence of hypertension, poor blood pressure control, incidence of risk factors, including the metabolic syndrome may partially explain the high cardiovascular and cerebrovascular mortality in the country.

**BILE ACIDS REPRESS CHOLESTEROL 7α-HYDROXYLASE GENE TRANSCRIPTION BY RECRUITING HISTONE DEACETYLASES**

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Elevation of plasma cholesterol is a well-known risk factor for atherosclerosis and coronary artery disease. Cholesterol is disposed mainly via its conversion to bile acids. This metabolic pathway is regulated at the level of the gene (*CYP7A1*) encoding cholesterol 7α-hydroxylase, which is an excellent target for novel hypocholesterolemic drugs. Here, we show that bile acids, the physiological regulators of *CYP7A1*, elicit the translocation of histone deacetylase-7 (HDAC7) to the nucleus. HDAC7 promotes the assembly of a repressive complex that determines the local deacetylation of histones, of the nuclear receptor hepatocyte nuclear factor-4α (HNF-4α and represses the transcription of *CYP7A1*. All these events are not mediated by the bile acid receptor FXR, as they occur in a short time frame, when the FXR-dependent upregulation of the repressor Small Heterodimer Partner (SHP) is still undetectable. Valproic acid (VPA), a known inhibitor of HDAC activity, prevents the bile acid-mediated repression of *CYP7A1* transcription by restoring the transcription machinery to the active state. *In vivo* administration of VPA increases *Cyp7a1* mRNA by antagonizing the feedback-regulation by endogenous bile salts corroborating the results obtained in *in vitro* experiments. Our study highlights the importance of the FXR/SHP-independent pathway of feedback-regulation of *CYP7A1* transcription and identifies a novel molecular target for effective treatment of hypercholesterolemia.
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**GENE-SELECTIVE MODULATION BY A SYNTHETIC OXYSTEROL LIGAND OF THE LIVER X RECEPTOR.**

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Liver X nuclear receptors (LXRs) play key roles in regulation of cholesterol homeostasis by limiting cholesterol accumulation in macrophages within arterial wall lesion sites by a mechanism that includes upregulation of ATP-binding cassette transporters (ABCs). These atheroprotective properties distinguish LXRs as potential targets for pharmaceutical intervention in cardiovascular disease. However, their associated activity for promoting lipogenesis and triglyceride accretion through activation of sterol-response element binding protein expression (SREBP-1c), represents a potential proatherogenic liability.

A newly characterized synthetic oxysterol, *N,N*-dimethyl-3β-hydroxycholenamide (DMHCA), represents a gene-selective LXR modulator that mediates potent transcriptional activation of ABCA1 gene expression while exhibiting minimal effects on SREBP-1c both *in vitro* and *in vivo* in mice. DMHCA has the potential to stimulate cholesterol transport through upregulation of LXR target genes, including ABCA1, in liver, small intestine and peritoneal macrophages. When compared with known nonsteroidal LXR agonists, however, DMHCA exhibits only limited activity for increasing hepatic SREBP-1c mRNA and does not alter circulating plasma triglycerides. Cell-based studies also indicate that DMHCA enhances cholesterol efflux in macrophages and suggest a mechanism whereby this selective modulator can potentially inhibit cholesterol accumulation. DMHCA and related gene-selective ligands of LXR may have application to the study and treatment of atherosclerosis.

## REGULATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α TRANSCRIPTIONAL ACTIVITY BY p38 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY

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Peroxisome proliferator-activated receptor alpha (PPARα) is a nuclear receptor involved in several physiological processes such as lipid and lipoprotein metabolism, glucose homeostasis and inflammatory response. In addition to ligand-induced transcriptional activity, PPARα is also regulated by its phosphorylation status. In the liver, PPARα is phosphorylated by kinases such as ERK mitogen-activated protein kinases (MAPK), cAMP-activated protein kinase (PKA) and protein kinase C (PKC). The aim of this work was to examine the effect of the p38 MAPK on PPARα transcriptional activity. First of all, we showed that in COS7 cells, anisomycin, a p38 MAPK activator, inhibited, in a ligand independent manner, the PPARα transcriptional activity. This inhibition was also found when a p38 MAPK was transfected alone or with a constitutively active MKK6, the specific p38 MAPK kinase. Interestingly, PPARα and p38 MAPK did not interact directly, but through a molecular adaptator, the zeta PKC-interacting protein (ZIP). When the interaction between p38 MAPK and ZIP has already been demonstrated, we showed that anisomycin led to the translocation of ZIP inside the nucleus. Using a single hybrid system, we found that ZIP was able to enhance the PPARα transcriptional activity. Moreover, using immunoprecipitation assays, we found a trimeric interaction between PPARα, p38 MAPK and ZIP. When analyzing the effect of mutation of putative MAPK phosphorylated sites in PPARα, our results suggested that other kinases, such as AMP-activated protein kinase (AMPK) or jun N-terminal kinase (JNK), could be involved in the regulation of PPARα transcriptional activity. Secondly, in H4IIE hepatoma cells, p38 MAPK activation by anisomycin decreased the endogenous expression of a PPARα target gene, the carnitine palmitoyltransferase I (CPTI). Finally, ZIP did not enhance the inhibitory effect of p38 MAPK neither on PPARα transcriptional activity nor on the endogenous expression of CPTI. In conclusion, we showed that the p38 MAPK inhibited PPARα transcriptional activity through a trimeric interaction between the p38 MAPK, ZIP and PPARα.

## EFFECT OF PPARα RECEPTOR AGONIST ON SPHINGOMYELIN PATHWAY OF SIGNAL TRANSDUCTION IN REGENERATING RAT LIVER.

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The family of PPAR receptors is involved in the regulation of lipid metabolism and cell cycle. Activation of PPARα isoform increases rate of cellular fatty acids degradation and induces hepatocyte proliferation in rat liver. Sphingomyelin pathway of signal transduction regulates cell cycle through release of lipid second messengers leading to apoptosis (ceramide, sphingosine) or proliferation (sphingosine-1-phosphate ; S1P) of the cells. The aim of the present study was to investigate the influence of bezafibrate (PPARα agonist) on second messengers' levels of sphingomyelin pathway in regenerating rat liver after partial hepatectomy. Three groups of Wistar rats were used in the experiment: a) control; b) subjected to partial hepatectomy; c) treated with bezafibrate (7,5mg/100g per day) by an oral gavage with subsequent partial hepatectomy. Sphingomyelin and ceramide were isolated by the means of thin layer chromatography. The content of various fatty acids in these two compounds was measured by gas-liquid chromatography. Cellular levels of sphingosine, sphinganine and sphingosine-1-phosphate were examined by High Performance Liquid Chromatography. It has been found that bezafibrate considerably lowered cellular levels of both sphingomyelin and ceramide in regenerating rat liver. Activation of hepatocyte proliferation was connected with significant increase of S1P vs. the control group. Bezafibrate decreased cellular levels of S1P in regenerating rat liver. Similar effect was found in case of sphingosine and sphinganine levels. It is concluded that bezafibrate can alter hepatocyte proliferation through the influence on the second messengers of sphingomyelin pathway.

## LIVER X RECEPTOR α (LXRα) mRNA LEVELS INDUCED BY PITAVASTATIN IN HEPG2 CELLS

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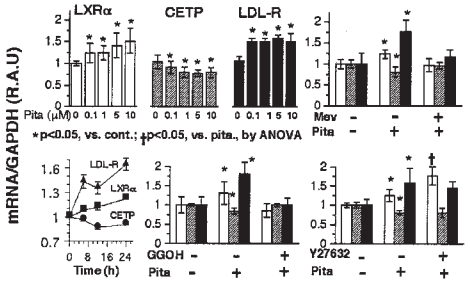
Background: To clarify the mechanism by which pitavastatin, a so-called super statin, decreases LDL and increases HDL levels, we examined the effects of pitavastatin on mRNA levels of CETP, LXRα, and LDL-R in HepG2 cells. Methods: Cell total RNA was extracted after drug treatment and mRNA levels were quantified by RT- real time PCR. Results: Pitavastatin decreased mRNA levels of CETP and increased those of LXRα and LDL-R (Fig.). Mevalonate and geranylgeraniol reversed the effects of pitavastatin on the expression of these genes. Inhibition of Rho-kinase by Y27632 enhanced the inductive effects of pitavastatin on LXRα mRNA levels, but not affected the effects of pitavastatin on CETP and LDL-R.

Conclusion: Suppression of CETP and induction of LXRα and LDL-R may contribute to the potent LDL-C-lowering and HDL-C-increasing effects of pitavastatin.




## IDENTIFICATION OF A NEW TYPE OF HYDROXYSTEROID DEHYDROGENASE AS A MAJOR PPARα-REGULATED GENE IN MOUSE INTESTINE

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It is now well accepted that PPARα plays an important role in lipid catabolism in the liver by up-regulating the expression of a variety of genes that encode proteins involved in fatty acid transport, β-oxidation and lipoprotein metabolism. However, these studies have been carried out using rodents over-expressing PPARα in the liver and strong synthetic PPARα agonists. Thus there is a possibility that our knowledge on the role of PPARα in lipid metabolism is biased against its extra-hepatic functions. In this study, we examined the PPARα agonist-induced proteins in the intestine, another important organ for lipid metabolism, to obtain a new aspect of the roles of PPARα. To detect the proteins whose expression levels were markedly altered by administration of a PPARα ligand, the post nuclear fractions of the intestine were prepared from wild and PPARα-null mice fed a control or Wy14,643-containing diet for five days. The proteins were compared by SDS-PAGE analysis and the Wy-induced proteins were identified by peptide mass fingerprinting followed by Northern blot analysis using their cDNAs. A mostly induced protein was identified as a new type of 17β-hydroxysteroid dehydrogenase. Its very rapid induction by various agonists was most efficient in the intestine. These data suggest new roles of both PPARα and 17β-hydroxysteroid dehydrogenase in sterol metabolism and/or detoxification in the intestine.

**IDENTIFICATION OF A NEW LIGAND FOR PEROXISOME PROLIFERATOR ACTIVATED RECEPTORα AND γ, A LEAD COMPOUND FOR THE THERAPY OF DIABETES AND OBESITY.**

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Peroxisome Proliferator-activated Receptors (PPARs) are ligand-activated transcription factors that control lipid and glucose homeostasis; therefore they play a central role in cardiovascular diseases, obesity and diabetes. There is significant interest in developing new specific ligands for these receptors, therefore aim of this study was to find novel compounds able to activate both PPARα and PPARγ. MS39, a chiral dual PPARα/γ agonist previously analyzed in our laboratory, was used as lead compound. In cell-based transactivation assay we characterized the structure-activity relationship of the new molecules. To better define the biological properties we studied the differentiation of 3T3-L1 murine adipocytes. All the analyzed compounds activate both PPARα and PPAR γ in transactivation assay and induce lipid accumulation in 3T3-L1 adipocytes. Moreover, these new dual agonists regulate the expression of genes involved in adipocyte differentiation and in triglyceride metabolism in liver, confirming the ability to activate PPARα and γ observed in transactivation assay. Our data demonstrate that LT160 is the most active dual compound because it activates both receptors with EC50 1nM on PPARα and 40 nM on PPARγ. These results indicate that LT160 is a candidate for the development of new dual PPAR agonists that could be used in diabetes, hyperlipidemia and obesity.

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**THE IN VIVO EFFECTS OF WY-14643 ON LIPID METABOLISM IN THE RAT HEART**

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The aim of the study was to examine the effects of PPARα ligand – WY-14643 on the content and composition of different lipid fractions and metabolism of sphingolipids in the rat heart. The experiments were carried out on male Wistar rats fed with a standard diet for rodents. The animals were divided into 2 groups: control and treated with WY-14643 (3 mg/kg/d) for the period of two weeks. Samples of the left ventricle were pulverized and lipids were then extracted with chloroform/methanol (2:1 v/v). Phospholipids (PH), sphingomyelin (SM), ceramide (CER), triacylglycerols (TG), diacylglycerols (DG) and free fatty acids (FFA) were then isolated by means of thin layer chromatography. Fourteen different fatty acid residues were identified and quantified in each lipid fraction by means of gas-liquid chromatography. The content of sphingosine (SO), sphinganine (SA) and sphingosine-1-phosphate (SO-1P) was measured by the use of a HPLC system equipped with a fluorescence detector. The activity of acid, neutral and alkaline ceramidase was determined by a radioisotopic method with the use of radiolabelled substrate ($^{14}$C-palmitoyl-sphingosine). WY-14643 increased the content of PH and TG and markedly lowered the amount of DG and FFA in the heart. The level of SM and CER remained unchanged. The ratio of saturated to unsaturated fatty acids in the hearts of WY-14643 treated animals was significantly lower in all measured lipid fractions with the exception of FFA and CER. The administration of WY-14643 induced small, but significant decrease in the content of SO and SA, the level of SO-1P was unaltered. WY-14643 didn't affect the activity of any of the examined ceramidases. It is concluded that WY-14643 exerts broad effects on lipid profile of the rat heart in vivo. It also affects the metabolism of sphingolipids in this organ.

**PROTEOMIC ANALYSES OF OBESE AND DYSLIPIDEMIC ZUCKER RATS TREATED WITH ACTIVATORS OF THE NUCLEAR RECEPTORS PPARα AND PPARγ.**

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Peroxisome proliferator activated receptors (PPAR´s), a family of nuclear hormone receptors, play a very important role in the regulation of cellular metabolism. Understanding the mechanisms of action of the PPAR activators is therefore of particular importance from the dyslipidemia disease area perspective. To improve our knowledge of how the PPAR activators correct fatty-acid metabolism disorders and increase the sensitivity to insulin, our group has utilized the proteomics technique. One animal model used is the obese and dyslipidemic Zucker rat, which have elevated plasma lipid levels. We treated these rats with either the PPARα-activator WY14,643 or the PPARγ-activator darglitazone and the resulting protein regulation in skeletal muscle, liver and white adipose tissues were studied.

**DIFFERENTIAL EFFECTS OF SYNTHETIC LXR AGONISTS ON PLASMA LIPOPROTEINS, INFLAMMATORY MARKERS, GENE EXPRESSION AND ATHEROSCLEROTIC LESION IN APOE KNOCK OUT MICE**

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ApoE knockout mice maintained on a high fat western diet were treated with 10 mg/kg/day T0901317 or GW3965 for 12 weeks and blood samples were taken at 2, 5, 8 and 12 week time points to quantitate serum cholesterol, triglycerides, lipoproteins and haptoglobin (an inflammatory marker). At the end of 12 weeks, the animals were sacrificed and liver and duodenum were removed for gene expression studies. Aortic arches and hearts were removed for lesion analysis. Both T0901317 and GW3965 decreased serum cholesterol levels at all time points, but the decrease with GW3965 was greater than T0901317. While, GW3965 had no effect on serum triglyceride levels, T0901317 caused a significant increase (5-7-fold) in triglyceride levels at all time points. Only GW3965 had a significant inhibitory effect on VLDL levels at all time points whereas both T0901317 and GW3965 decreased serum LDL at all time points. Only T0901317 had a significant inhibitory effect on serum haptoglobin at all time points whereas GW3965 had no effect. Neither T0901317 nor GW3965 had any effect on ABCA1 expression in the livers of these animals. Additionally, T0901317 treatment resulted in a significant increase in liver SREBP1c expression whereas GW3965 had no effect. The increase in liver SREBP1c expression by T0901317 agrees with its effect on serum triglycerides. Both ABCA1 and SREBP1c were induced in the duodenum by both compounds. Both T0901317 and GW3965 inhibited aortic arch and aortic root lesion formation although the inhibition by GW3965 was much greater than that observed with T0901317 (80% vs. 30% respectively). The data indicate that both LXR agonists display different effects in this model of atherosclerosis.

## THE METABOLIC EFFECT OF PPARα, PPARγ AND PPARδ SELECTIVE AGONISTS IN TYPE 2 DIABETIC DB/DB MICE

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The peroxisome proliferator activated receptors (PPAR) are transcription factors belonging to the nuclear receptor super family. Three sub types have been identified: PPARα, PPARγ and PPARδ. Fibrates, PPARα agonist, have for several decades been used for the treatment of dyslipidemia. Insulin sensitizers, PPARγ agonists, are newer drugs used for treatment of type 2 diabetes. The physiological role of PPARδ is still unclear but animal data indicate that activation of PPARδ attenuate dyslipidemia and insulin resistance. The aim of this study was to examine the metabolic effects in db/db mice after 12 weeks treatment with the selective PPARδ agonist GW501516 in comparison with fenofibrate (PPARα) and rosiglitazone (PPARγ). In addition was body fat determined using DEXA and QCT scanning. The head to head comparison was made in db/db mice, 5 weeks old at study start. The mice were dosed orally twice daily for 12 weeks with either vehicle (10 ml/kg), fenofibrate (2 x 50 mg/kg), rosiglitazone (2 x 5 mg/kg) or GW501516 (2 x 5 mg/kg). Body weight and 24 hours food intake was recorded once a week and plasma was analysed at the end of the treatment period. All three compounds lowered non-fasting plasma glucose and insulin levels but only fenofibrate and GW501516 lowered plasma triglycerides and increased HDL cholesterol as shown in the table:

| | GLU (mM) | INS (pM) | HDL/TC | TG (mM) |
|---|---|---|---|---|
| db/+ veh. | 9.52±2.02 | 66±42 | 0.74±0.06 | 1.15±0.53 |
| db/db veh. | 18.09±6.54 | 2052±989 | 0.85±0.02 | 1.39±0.14 |
| Feno | 12.81±2.90* | 808±462** | 0.91±0.02** | 0.67±0.10** |
| Rosi | 10.37±3.62 | 320±193** | 0.81±0.09 | 1.30±0.27 |
| GW | 13.13±2.18* | 979±180** | 0.94±0.03** | 1.01±0.19** |

These results show that long term PPARδ activation correct dyslipidemia, hyperinsulinemia and hyperglycemia in type 2 diabetic db/db mice.

## ISOHUMULONES, BITTER COMPOUNDS IN BEER, ACTIVATE HEPATIC PPAR ALPHA AND ATTENUATE ATHEROSCLEROSIS

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Isohumulones, the major bitter compounds in beer, have dual agonist activity for peroxisome proliferator-activated receptor (PPAR) α and γ *in vitro*. In this study, we fed Iso-hop extracts (IHE) mainly containing isohumulones to wild type (129SV) and PPARα deficient mice, and analyzed their biochemical parameters and hepatic gene expression profiles. Similar to a PPARα agonist, fenofibrate, IHE up-regulated genes for fatty acid oxidation, and ameliorated blood lipid status. However, these effects were abolished in PPARα deficient mice, suggesting that the activation of PPARα is required for the modulation by IHE. We further analyzed the effect of IHE ingestion in apolipoprotein E deficient mice. Although IHE did not ameliorate the blood lipid status, it significantly reduced the atherosclerotic lesions and thicknesses at early stage with a significant reduction in blood levels of IL-6, sICAM-1, and sVCAM-1. These results suggested that the anti-inflammatory effects of IHE are at least partly responsible for the attenuation. The agonistic activity for PPARα and anti-inflammatory effects of IHE may be beneficial for the prevention of lifestyle related diseases such as atherosclerosis.

## PPARDELTA DOES NOT INFLUENCE CHOLESTEROL METABOLISM IN SUBJECTS WITH TYPE 2 DIABETES MELLITUS OR IN HEALTHY CONTROLS

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Purpose of the present study was to examine whether the +294T/C peroxisome proliferator activated receptor δ (PPARδ) polymorphism, which ahs been shown to play a role in cholesterol (C) metabolism in animal models, is associated with altered cholesterol levels in healthy controls and in subjects with type 2 diabetes mellitus (DM-2).We determined by PCR the aforementioned polymorphism in 402 patients with DM-2 (230 men and 172 women) and in 436 healthy controls (248 men and 188 women) from the LIANCO Study (Lipid-Analytic-Cologne). The genotype distribution was not different between DM-2 and controls (65.6 vs. 66.7% PPARδ +294TT, 30.5 vs. 29.4% PPaRδ +294TC, 3.9 vs. 4.9% PPARδ +294CC). There was no difference in the LDL-C, HDL-C or triglyceride (TG) concentrations between individuals with DM-2 carrying the rare PPARδ +294C allele, either heterozygotes (LDL-C 157 ± 80, HDL-C 54 ± 17, TG 273 ± 247 mg/dl) or homozygotes (LDL-C 149 ± 44, HDL-C 59 ± 21, TG 197 ± 110) and non-carriers (LDL-C 156 ± 49, HDL-C 56 ± 18, TG 242 ± 225 mg/dl). The same lack of association was observed in the control group (data not shown). There was no difference between men and women. No associations were found between the polymorphism and body mass index. In summary, the present study shows that the PPARδ +294T/C polymorphism has no influence on plasma lipoprotein concentrations either in healthy subjects or in patients with DM-2.

## IN VITRO TRANSCRIPTIONAL REGULATION OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN 9

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**Introduction:** Proprotein convertase subtilisin/kexin 9 (PCSK9) has been identified as a third locus for Autosomal Dominant Hypercholesterolemia (ADH). Screening for genetic variation in PCSK9 revealed a promoter variant –245 G→T, found exclusively in three non-LDLR/non-apoB linked ADH patients. To assess the role of PCSK9 in lipoprotein metabolism, we studied the characteristics of the promoter region of PCSK9. **Methods:** MatInspector was used to search for functional sequence motifs. Several promoter constructs were cloned into a luciferase reporter gene and transfected into HepG2 cells, cultured under different conditions (lipid deficient serum, lovastatin, and the LXR agonist TO901317). Activity was measured using a dual luciferase activity assay. **Results:** MatInspector analysis revealed a potential LXR binding site, located between –249 to -239, which was disrupted in the presence of the promoter variant and a SRE located between –349 to -335. The -245T mutation did not affect transcriptional activity under any of the conditions tested. Transcriptional activity of wild-type and mutant promoter was up-regulated 3-fold by TO901317 and 3.8-fold by lovastatin. Constructs with and without the SRE reduced activity by 78% compared to the wild-type promoter construct. The construct containing the SRE had 60% higher activity in the presence of lovastatin compared to the construct without the SRE. **Conclusion:** Although the promoter variant identified in three non-LDLR/non-apoB linked ADH patients was non-functional and therefore not related to the ADH phenotype, PCSK9 proved to be under control of cholesterol responsive elements. The presence of a functional SRE and a LXR agonist responsive site indicates that PCSK9 plays a role in lipoprotein metabolism.

## GEMFIBROZIL IN THE TREATMENT OF DYSLIPIDEMIA: A 18-YEAR MORTALITY FOLLOW-UP OF THE HELSINKI HEART STUDY

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**Background** The Helsinki Heart Study was a randomized, double–blind primary prevention trial in middle-aged males to test the efficacy of gemfibrozil in the prevention of coronary heart disease (CHD). The five year trial ended in 1987 and showed a 34% reduction of CHD in the gemfibrozil (G) group compared to placebo (P). After the trial the participants were offered free gemfibrozil treatment until the end of 1995. Approximately 2/3 in both treatment groups choose to continue on gemfibrozil. In this intention–to-treat-based mortality follow-up of the initial treatment groups we studied the effect of a five year earlier start of gemfibrozil treatment.
**Methods and Results** Using Cox proportional hazards models to study the CHD mortality in the the two groups we found that the CHD mortality was 23% lower ( p=0.05) in the G group than in the P group. Subgroup analyses revealed that the greatest differences between the G and P groups were seen among those with high body mass index (BMI) or high triglyceride (TG) level or low HDL-cholesterol (HDL-C) – or combinations of these factors. Among those with both BMI and TG in the highest tertiles of distributions, the CHD mortality was 71% lower (p=0.0001) in the G group than in the corresponding P subgroup. The all cause and cancer mortality were also lower in this subgroup by 33% (p=0.03) and 35% (p=NS).
**Conclusions** Our findings suggest that those with dyslipidemia, related to insulin resistance benefit most from early treatment with gemfibrozil.

## CHARACTERIZATION OF THE HYPOLIPIDEMIC EFFECTS OF FENOFIBRATE IN HYPERLIPIDEMIA INDUCED BY HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT AS COMPARED TO FAMILIAL COMBINED HYPERLIPIDEMIA

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Highly active antiretroviral treatment (HAART) has profoundly improved the prognosis of HIV-infected patients. However, it often associates with marked metabolic alterations, including hyperlipidemia, which might affect long-term cardiovascular risk. The pathogenesis of such changes is largely unknown. AIM of this study was to investigate the effects of fibrate treatment in patients receiving HAART, in comparison with subjects with primary hyperlipidemia. METHODS. 17 HIV-infected patients receiving HAART with hypertriglyceridemia or combined hyperlipidemia and 38 patients with familial combined hyperlipidemia (FCH) and similar lipid phenotype were studied in basal conditions and after 2-3 month treatment with micronized fenofibrate (200 mg/d). Plasma lipid and lipoprotein/apolipoprotein profile were assayed. RESULTS. In FCH subjects, as expected, fenofibrate significantly ($p < 0.05$) reduced plasma total cholesterol (by 17%), triglyceride (by 61%), apo B (by 21%) with increases in HDL-cholesterol and apo AI. In HAART receiving patients only plasma triglyceride levels were significantly reduced (from 742±303, mean ± SD, to 528±385, $p < 0.05$ by paired *t* test). Changes in total and LDL-cholesterol were significantly correlated with changes of apo B levels in FCH but not in HAART patients. CONCLUSIONS. The metabolic effects of fenofibrate are markedly different in the two forms of hyperlipidemia. Data on the correlation between changes in lipid and apo B levels support the hypothesis that increased apo B production may play a causal role in FCH, whereas different mechanisms are likely to operate in HAART-related hyperlipidemia. However, micronized fenofibrate may be a useful tool in the management of HAART-related hyperlipidemia, possibly by mechanisms not involving changes in apo B production.

## A COMPARATIVE CROSSOVER STUDY OF THE EFFECTS OF FLUVASTATIN AND PRAVASTATIN (FP-COS) ON CIRCULATING AUTOANTIBODIES TO OXIDIZED LDL IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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This study compared the effects of fluvastatin and pravastatin on the in vivo oxidation of LDL in a crossover design to evaluate whether or not it is justified to switch between the two statins with regard to serum levels of lipids, lipoproteins, and apolipoproteins (apo), and circulating autoantibodies to oxidized LDL (OxLDL-Ab). Patients with hypercholesterolemia (n=46) were randomly assigned into groups who received fluvastatin (20 mg/d) or pravastatin (10 mg/d). After 3 months, they were crossed to receive the other statin for another 3 months. Circulating levels of OxLDL-Ab were measured by an OxLDL IgG ELISA test. Fluvastatin and pravastatin similarly decreased serum levels of total cholesterol (TC), LDL-C, and apo B, and increased HDL2-C levels. After crossover to the other statin, these lipid parameters were not further changed by either statin. Before crossover, circulating levels of OxLDL-Ab were decreased in patients with fluvastatin treatment, but not in those with pravastatin treatment. After switching from the other statin, both fluvastatin and pravastatin further decreased OxLDL-Ab levels. In conclusion, fluvastatin at 20 mg/d and pravastatin at 10 mg/d are interchangeable with regard to their efficacy in decreasing TC, LDL-C, and apo B levels and increasing HDL2-C levels. While both fluvastatin and pravastatin lowered circulating levels of OxLDL-Ab, pravastatin needed to be preceded by fluvastatin for its lowering effect to be detected.

## SIMVASTATIN AND CIPROFIBRAT COULD REDUCE LDL OXIDATION PROBABLY WITH DIFFERENT INFLUENCES

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The aim of the study was to assess the effects of lipids lowering treatment with Simvastatin or with Ciprofibrat on lipid profile and oxidation of LDL. 44 type 2 diabetics were in **group A**, 23 of them have been with previously diagnosed CHD. They had hypercholesterolemia and were treated with Simvastatin 20-40mg/day. In **group B** were 22 type 2 diabetics.They had hypertriglyceridemia and were treated with Ciprofibrat 100mg/day, 6 patients had CHD. Total, HDL and LDL cholesterol (Ch), triglycerides (TG) was analyzed by enzymatic methods. Apolipoprotein (Apo) AI, AII, B100, E,and Lp(a)as well as C reactive proteine ( CRP) were measured by nephelometry. Oxidased (Ox) LDL was measured by ELISA methods (Mercodia). In first group Total Ch was 7.54±0.26 mmol/l, LDL Ch: 5.10±0.29mmol/l and HDL Ch: 1.39±0.0.35mmol/l. TG were 2.25±0.27mmol/l. We calculated non-HDL Ch (6.18 ±0.29mmol/l) and TG/HDL Ch ratio (1.55±0.21). In group B Total Ch was 6.62±0,41 mmol/l, LDL Ch 3.61±0.29mmol/l, HDL Ch: 1.30±0.14 mmol/l and TG: 5.04±1.42 mmol/l. Non HDL Ch was 5.19±0.55 mmol/l. They had high TG/HDL Ch ratio 3.38±0.96. In both group Ox LDL was elevated, but CRP, was increased only in group A. After three months of treatment we found in both groups decrease of Ox LDL and CRP. In group A, OxLDL decreased from 146.44±16.79 to 109.87 IU/l ($p<0.05$) and in group B from 102.23±14.67 to 88.67±12.17 IU/l ($p<0.01$). In group A, patients treated with Simvastatin significantly decreased total Ch ($p<0.01$), LDL ($p<0.01$) and non HDL Ch ($p<0.01$) as well as apo B 100, apo E ($p<0,01$) and CRP from 9.94 ±2.61 to 6.23±1.16 ($p<0.05$). Decrease of Ox LDL is correlated with decrease of LDL ($p<0,01$), non HDL Ch ($p<0,05$), apoB ($p<0,05$) and apoE ($p<0.05$). In group B, patients treated with Ciprofibrat, we found decrease in TG ($p<0.01$) and total Ch ($p<0,05$), as well as TG/HDL Ch ratio (from 3.39±0.96 to 2.59±0.55; $p<0.01$), with increase in HDL Ch ($p<0.01$). We did not find other significant changes in group B. Simvastatin and Ciprofibrat significantly decreased Ox LDL, but with different pathway. Simvastatin predominantly had effect in decreasing of LDL Ch level. Ciprofibrat decreased oxidation of LDL by increasing HDL Ch. In spite of significant decrease in TG/ HDL Ch ratio, Ciprofibrat probably did not normalize small dense LDL level.

## COMPARISON OF THE EFFECTS OF ATORVASTATIN AND FENOFIBRATE ON APO-B-CONTAINING LIPOPROTEIN SUBFRACTIONS IN PATIENTS WITH MIXED DYSLIPIDEMIA

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It has been shown that LDL subfractions' profile may represent an independent risk factor for the development of cardiovascular disease. Although the impact of hypolipidemic drugs on the conventional lipid parameters has been extensively studied, their effects on the concentration and relative distribution of lipoprotein subfractions remain ill defined. We undertook the present study to evaluate and compare the effects of atorvastatin and fenofibrate on apolipoprotein B-containing lipoprotein subfractions. Forty-four patients with combined dyslipidemia were included. At the end of the dietary lead-in period patients were randomised to receive either atorvastatin (20mg, n=21) or fenofibrate (200mg, n=23). Serum lipids and lipoproteins and the concentrations of individual lipoprotein subfractions were determined at baseline as well as after 16 weeks of active treatment. Both drugs sufficiently reduced the concentrations of total and LDL cholesterol and triglycerides. In addition, fenofibrate increased the values of HDL cholesterol. Atorvastatin and fenofibrate significantly reduced the concentrations of VLDL+IDL as well as of total LDL. These reductions in total LDL mass were due to the reductions in the masses of all individual LDL subspecies. When the changes in the concentrations of LDL subfractions were compared with analysis of covariance taking into account the baseline values as a covariate, no significant differences were found among the two hypolipidemic drugs. Our results support the assumption that adequate doses of atorvastatin have the same effect as fenofibrate on LDL subfractions' metabolism, and thus raise important questions concerning the need of combination therapy in patients with mixed dyslipidemia.

## EFFECTS OF SIMVASTATIN AS A THERAPY IN HYPERLIPIDEMIA

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Dyslipidemia is a major risk factor for coronary heart disease. While some uncertainly exists about the clinical significance of improving high density lipoprotein cholesterol and triglyceride levels, large primary and secondary prevention studies aimed at lowering low-density lipoprotein cholesterol levels with statins have convincingly reduced coronary heart disease events and total mortality.
The aim of this study is to evaluate the concentration of serum lipids in patients with hyperlipidemia, before and after various therapy. We have analyzed 29 patients, and they have been treated with drugs Vasilip (Simvastatin) –22 patients and diet – 7 patients, in time from one month. As a control group, we included 30 healthy subjects. The laboratory methods were the routine enzymatic assays, and LDL-cholesterol calculated by Fridewald' s formula.
The research results after therapy with Vasilip (one month), show significant reduction in cholesterol 23.2% ($p<0.050$) and LDL-cholesterol 26.3%. Diet treatment produces reduction of the cholesterol, triglycerides, LDL-cholesterol and increasing of HDL-cholesterol, but it is no significant.
It can be concluded that treatment with Vasilip is more successful than diet treatment, because Vasilip is HMG CoA reductase inhibitor. The most recently developed class of drugs for lowering cholesterol, "statins" work inhibiting cholesterol production by the liver. Statin drugs are very effective for lowering LDL-cholesterol levels and have a moderate effect in raising HDL levels and lowering triglycerides.

## EFFECT OF SMALL DOSES OF ATORVASTATIN ON LIPID AND LIPOPROTEIN SUBFRACTION PROFILES IN CORONARY HEART DISEASE PATIENTS

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**Background:** A preponderance of small dense LDL is strongly associated with elevated CHD risk. Evidence from several studies suggests that additional to reducing overall CH-LDL, some statins can qualitatively change LDL subfraction profile.
**Methods:** The effects of small doses of atorvastatin on lipid and lipoprotein subfraction profiles were evaluated in 17 CHD patients with hypercholesterolemia (HCH) in simple open controlled study during 16 weeks (8 weeks - 2,5 mg/day, and then 8 weeks - 5 mg/day). The criteria for including patients into the study were CHD men and women at the age of 40-70, with blood TCH>6,0 mM/l. Atorvastatin effects on blood lipid profile were determined by enzymatic methods. Atorvastatin effects on various lipoprotein subfraction profiles (level and chemical composition of lipoprotein subfractions included CH, TG and apo-LP contents in them) were determined using small-angle X-ray scattering technology.
**Results:** In CHD patients atorvastatin significantly reduced TCH and CH-LDL levels at small doses (2,5 mg: -16% and -19%; 5 mg: -23% and -30%, $p<0,05$, respectively). Influence of small doses of atorvastatin on CH-HDL and TG blood levels was not revealed. At small doses, atorvastatin significantly reduced the levels of IDL and LDL particles, especially of small dense LDL (-45%, -13% and -26%, $p<0,05$, respectively), reduced CH and TG contents in small dense LDL (-25% and -31%, $p<0,05$, respectively).
**Conclusion:** Atorvastatin beneficially altered the atherogenic lipid and lipoprotein profiles in CHD patients with HCH. As expected, atorvastatin significantly reduced TCH and CH-LDL levels. Furthermore, atorvastatin significantly decreased the level of atherogenic small dense LDL.
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## GENETIC AND ENVIRONMENTAL FACTORS AFFECTING THE RESPONSE TO STATIN THERAPY IN PATIENTS WITH MOLECULARLY DEFINED FAMILIAL HYPERCHOLESTEROLEMIA

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Familial hypercholesterolemia (FH) is the most common inherited metabolic disease characterized by elevated serum levels of LDL-cholesterol (LDL-C) and ischaemic heart disease (IHD) early in life. Early diagnosis and treatment are essential to prevent premature atheromatosis in FH patients.
The aim of our study was the evaluation of the effects of genetic and environmental factors to the response to statin therapy in patients with molecularly defined FH.
Atorvastatin 20 mg/day was prescribed in 49 patients with molecularly defined heterozygous FH. Lipid profile was examined before and after 12 weeks of therapy. Apolipoprotein (apo) E, apo A-IV and cholesterol ester transfer protein (CETP) gene polymorphisms were examined using previously described protocols.
Stain therapy resulted in a decrement of 37% of LDL-C levels after 12 weeks of therapy. The % change in LDL-C was correlated with the initial LDL-C levels ($r=0.348$, $p=0.01$). The type of the LDL receptor (LDLR) also affected the response of heterozygous FH patients to statin therapy. In detail, heterozygotes sharing a type V mutation of the LDLR (the G1775A mutation, n=21) showed a higher percentage decrease in LDL-C after atorvastatin administration compared with patients sharing type II mutations (the G1646A and the C858A mutations, n=28) [49±9 vs 34±9%, $p=0.00$]. None of the other gene polymorphisms studied affected the lipid lowering effect of atorvastatin in patients with heterozygous FH. Moreover, age, sex, body mass index (BMI) and smoking habit did not affect the statin lipid lowering effect in these patients.
Our data point out that the type of the LDLR gene mutation affects the response of heterozygous FH patients to statin therapy. Specifically, patients with a type V mutation show higher percentage decrease in LDL-C levels after statin therapy compared with patients sharing type II mutations.

## USAGE OF A NEW EXTENDED-RELEASE FORMULATION FLUVASTATIN XL WITH AN IN PATIENTS WITH COMBINED HYPERLIPIDEMIA AND HIGH GLOBAL CORONARY RISK

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It is known that, fluvastatin is the only statin with a hepatic saturable potential at doses between 20-40 mg, resulting in higher doses essentially spilling into the peripheral circulation and thereby losing the expected additional LDL. Fluvastatin XL is a modified release 80 mg dose that achieves a 33% to 36% LDL reduction, which is greater than the 31% reduction of 40 mg of immediate release formulation given twice a day. The purpose of the present study was to evaluate lipid-modulating effect of Fluvastatin XL and its influence to global coronary risk.

The study group comprised 20 patients with combined hyperlipidemia (IIb type) and high global coronary risk which received fluvastatin XL in doses 80 mg daily during 24 week. SBP and DBP levels, serum total CH, LDL CH, triglycerides, HDL CH, fasting glucose were measured before and after treatment. Global coronary risk (in %) was calculated according to PROCAM model, which is determined by non-modifying (age, sex, family history of CHD) and modifying (hypertension, cigarette smoking, LDL and HDL cholesterol, triglycerides levels) CHD risk factors.

At baseline, patients had following lipid disturbances: total CH 288±10 mg/dl, LDL CH mg/dl 210±7 mg/dl, TG 200±15 mg/dl and HDL CH 38±1 mg/dl, which accompanied with high global coronary risk - 36%, which two-fold exceeded threshold level. After 4 week of treatment total CH concentration reduced by 34%, LDL CH - 44% and triglycerides - 32%, while HDL CH increased by 16%. At the end point of trial this tendency was preserved, and reduction in total CH was achieved up to 192±8 mg/dl, in LDL CH - 118±8 mg/dl, in triglycerides - 136±18 mg/dl, and in HDL CH - 44±2 mg/dl. The LDL CH goal were achieved by 78% of patients. As a result of therapy with fluvastatin XL global coronary risk decreased by – 60%, and comprised 16%.

Thus, in patients with combined hyperlipidemia (IIb type) and high global coronary risk monotherapy with fluvastatin XL in daily dose 80 mg benefit decreased the serum LDL CH, triglycerides, and increased HDL CH which accompanied by significant reduction of the risk of CHD events .

## LDL-C/HDL-C RATIO IN PATIENTS WITH CORONARY ARTERY DISEASE AND LOW HDL-C: THE RADAR STUDY

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Low levels of HDL-C are a risk factor for coronary artery disease (CAD) and the LDL-C/HDL-C ratio is a powerful predictor of clinical events. RADAR was a randomised, multicentre, parallel-group, study that compared the effect of rosuvastatin (RSV) with atorvastatin (ATV) on lipid levels in patients with CAD and low HDL-C levels. Following dietary lead-in, 461 patients aged 40–80 years with established atherosclerotic disease and HDL-C <1 mmol/l were randomised to receive RSV 10 mg (n=230) or ATV 20 mg (n=231). Patients were forced-titrated at 6 weeks (RSV 20 mg, ATV 40 mg) and 12 weeks (RSV 40 mg, ATV 80 mg) (18 weeks' total treatment). Baseline LDL-C (mean ± SD) was 3.6 ± 1.2 mmol/l (RSV) and 3.7 ± 1.3 mmol/l (ATV), and HDL-C, 0.8 ± 0.1 mmol/l (RSV) and 0.8 ± 0.1 mmol/l (ATV). RSV reduced LDL-C and the LDL-C/HDL-C ratio (table) more than ATV. Furthermore, significantly more patients achieved 2003 European LDL-C goals (<2.5 mmol/l) with RSV compared with ATV after 18 weeks (RSV 93.9% vs ATV 85.3%; p<0.01). Both treatments were well tolerated. In conclusion, RSV is more effective than ATV at reducing LDL-C and improving the LDL-C/HDL-C ratio, and thereby enables more patients with CAD and low HDL-C levels to achieve their LDL-C goal.

| | Least squares mean change from baseline (%) | | | | | |
|---|---|---|---|---|---|---|
| | 6 Weeks | | 12 Weeks | | 18 Weeks | |
| | RSV 10 mg | ATV 20 mg | RSV 20 mg | ATV 40 mg | RSV 40 mg | ATV 80 mg |
| LDL-C/HDL-C ratio | –47.0[a] | –41.9 | –53.0[b] | –47.9 | –57.3[c] | –49.6 |
| LDL-C | –44.0[a] | –38.4 | –50.4[b] | –45.1 | –55.3[c] | –48.1 |
| TC | –37.4[d] | –32.5 | –41.1[c] | –37.3 | –44.7[d] | –39.5 |
| HDL-C | +3.9 | +4.1 | +5.5 | +3.1 | +4.7 | +2.7 |

[a]p<0.05; [b]p<0.01; [c]p<0.001; ; [d]p<0.0001 vs ATV at the same time point

## LYMPHOCYTE LDL-RECEPTOR ACTIVITY AND PLASMA LDL-C LOWERING BY ATORVASTATIN IN PATIENTS WITH FH AND FCHL

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Relationship between lymphocyte LDL-R activities and decreases in plasma LDL-C was investigated in patients with non-familial hypercholesterolemia (NFHC) (N=10), FH (N=13), and FCHL (N=14). Treatment duration was 12 months. LDL-R activities were 110±34 (M ±SD)%, 71±15%, and 102±22% in patients with NFHC, FH, and FCHL, respectively. Plasma levels of LDL-C decreased from 198± 25mg/dL, 330±53mg/dL,and 261±44mg/dL to 121±29mg/dL, 153± 28mg/dL, and 132±30mg/dL in patients with NFHC, FH, and FCHL. The doses of atorvastatin were minimum (10±0 mg/day) for patients with NFHC, maximum (40 ±0 mg/day) for patients with FH, and 26± 13 mg/day for patients with FCHL. The absolute decrease in plasma LDL-C was greatest in patients with FH and next in those with FCHL. Atorvastatin is very effective for patients with FCHL. Is is probably due to the inhibitory effects on hepatic production of TG-rich lipoproteins.

## EFFECTS OF ATORVASTATIN ON PLASMA LEVELS OF THREE ULTRACENTRIFUGALLY SEPARATED LDL SUBFRACTIONS IN PATIENTS WITH FH AND FCHL

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The disributions of LDL subfractions are different between patient with FH and those with FCHL. Effects of atorvastatin on plama LDL subfractions were compared among patients with non-familial hypercholesterolemia (NFHC)(N=10), FH (N=13), and FCHL (14). Treatment duration was 12 months. Plasma LDL1-C, LDL2-C, and LDL3-C changed from 56±11 mg/dL, 78±15 mg/dL, and 29±12 mg/dL to 28±7 mg/dL, 43±9 mg/dL, and 19±7 mg/dL in patients with NFHC, respectively. Plasma levels of LDL1-C, LDL2-C, and LDL3-C decreased from 78±36 mg/dL, 136±40 mg/dL, and 40±25 mg/dL to 37±18 mg/dL, 61±14 mg/dL, and 23±17 mg/dL in patients with FH. Plasma levels of LDL1-C, LDL2-C, and LDL3-C decreased from 69±34 mg/dL, 88±27 mg/dL, and 42±12 mg/dL to 30±14 mg/dL, 50±17 mg/dL, and 23 ± 11 mg/dL in patients with FCHL, respectively. Plasma 3 subfractions of LDL equally decreased in each of 3 groups.

## FLUVASTATIN ER FORMULATION IN BOTH GENDERS REDUCES CHOLESTEROL SYNTHESIS INDEPENDENTLY OF DAYTIME OF ADMINISTRATION

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Statins inhibit one of the rate-limiting steps in cholesterol synthesis, the conversion of HMG-CoA to mevalonate by HMG-CoA reductase. Statins are usually taken in the evening as HMG-CoA reductase activity is high during nighttime. This recommendation might not apply if a statin is given as extended release (ER) drug formulation. The present study investigated the influence of daytime of drug intake on plasma and urine concentrations of mevalonic acid in females and males by fluvastatin (80 mg ER).
This was a randomized, 2 period cross-over study with single dose administration during each period and a wash-out period of 6-8 days. After a dietary run-in phase of 1-2 weeks, 26 hypercholesterolemic patients (13 female/13 male) were randomly assigned to treatment. At baseline, patients (means of all) had TC 243 mg/dl, LDL-C 161 mg/dl, HDL-C 54 mg/dl, TGs 167 mg/dl, and creatinine 1.16 g/24 h. Excretion of 24-h mevalonic acid was 204.9 ± 68.1 µg/g creatinine.
After a single dose of fluvastatin mean urine values of mevalonate were significantly reduced to 129.8 ± 66.2 µg/g (evening) and to 118.7 ± 34.3 µg/g (morning; ns between groups). Thus, giving fluvastatin in the morning was as effective as in the evening and reduced mevalonate excretion by about 39 %. In comparison, females in general showed slightly higher values than males.
Compared to baseline, also plasma mevalonate concentrations were decreased by fluvastatin, resulting in similar allover 24-h profiles of the morning and the evening dosage with generally lower values in males.
Fluvastatin pharmacokinetics of both periods were comparable, with a trend to higher plasma concentrations for several hours following evening dosage. Systemic bioavailability was markedly higer in females than in males.
This study indicates that in both genders fluvastatin ER inhibits cholesterol biosynthesis regardless of daytime of administration.

## APOE PHENOTYPE-SENSITIVE LIPID RESPONSE TO FLUVASTATIN TREATMENT AMONG PATIENTS WITH HYPERCHOLESTEROLEMIA

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67 male patients (33 with apoE3/3 phenotype, E3 group; 23 with 2/2 or 2/3, E2+ group; 11 with 4/4 or 4/3, E4+ group) differing widely in triglyceride (TG) content but mainly with moderate or high hypercholesterolemia, received daily 20-40 mg of fluvastatin for 12 weeks. The levels of TG, Cholesterol (Chol), LDL-Chol (LDL-C) and HDL-Chol (HDL-C) were measured after 0, 4, 8 and 12 weeks. Lipid percentage changes (Δ) were not associated with apoE phenotype. The relative change in TG content after 4 weeks ($\Delta TG_{4/0}$) tended to be positive for E4+, not to change for E3 and to be negative for E2+ groups and became negative and more evident after 8 and 12 (-7/-10%) weeks for all three groups. Cholesterol decreased by 14% after 12 weeks and HDL-cholesterol increased by 14-16% after 12 weeks monotonously for all three groups. TG, Chol, LDL-C changes associated positively, while negatively for HDL-C change, with the basal values for the same lipid for all three groups. The positive ΔTG values occured at low basal triglyceride ($TG_0$) level and became negative at $TG_0$ > 1.6-1.9 mM. For E3 and E4+ groups, only a single parameter contributed significantly into a variation of lipid changes. For E2+ group, $TG_0$ and $Chol_0$ content contributed oppositely into $\Delta TG_{12/0}$, both positively into $\Delta Chol_{8/0}$; $Chol_0$ and HDL-$C_0$ both negatively contributed into $\Delta$HDL-$C_{12/0}$. HDL-$C_0$ content contributed oppositely into LDL-C variability for E2+ and E4+ groups. Three effects in combination seem to contribute differently into lipid response among patients with different apoE phenotype, i.e. the inhibition of hepatic and lipoprotein lipase activities, the competition between TG-rich and low density lipoproteins for the LDL receptor and the accumulation of IDL particles in patients bearing ε2 allele.

## EFFICACY AND SAFETY OF LONG-TERM ATORVASTATIN TREATMENT IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE

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The aim of the study was to evaluate the efficacy and safety of long-term atorvastatin treatment in patients (pts) with hypercholesterolemia and stable coronary artery disease (CAD). Methods: We studied 100 pts with stable CAD, a relatively normal left ventricular function, symptomatic or mild to moderate angina, and low-density lipoprotein cholesterol (LDL-C) of at least 160 mg/dl and serum level of triglycerides of less than 300mg/dl, despite cholesterol lowering diet. Pts mean age was 55±4 years. Pts were randomly assigned to receive atorvastatin (20 mg/dl) or placebo. Background therapy was similar for group and consisted of nitrates, beta-blockers, ACE inhibitors, anticoagulants. Results: At 1 year in the atorvastatin group the average percent changes from baseline were as follows: total cholesterol −31.2% ($p<0.001$), LDL-C −41.6% ($p<0.0001$), high density lipoprotein cholesterol +14.39% ($p<0.005$), triglycerides −25.09% ($p<0.001$). In the placebo group the corresponding changes were: +2%; +1%; +2% (all p=NS). After 1 year of atorvastatin treatment the number of effort of angina attacks per day decreased by 80.9% ($p<0.0001$) and the consumption of nitroglycerine by 78.4% ($p<0.0001$). Atorvastatin therapy has been shown to reduce significantly the incidence of cardiovascular events, overall mortality and the need for revascularization. Therapy with atorvastatin was well tolerated. Adverse events occurred with similar frequency in the groups. The syndrome of drug-induced myopathy was not observed in any pts, although no pts receiving atorvastatin was discontinued for an increased transaminase levels. Conclusion: The results of the study suggest that atorvastatin is a powerful and well tolerated agent for the treatment of pts with stable CAD.

## LIPID-LOWERING AND SOME PLEIOTROPIC EFFECTS OF VASILIP (SIMVASTATIN) IN SUBJECTS WITH CORONARY HEART DISEASE

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The aim of study was to evaluate lipid and non-lipid effects of treatment with HMG-CoA reductase inhibitor simvastatin (Vasilip-KRKA,Slovenia) in patients with coronary heart disease (CHD). 92 patients were studied (84 males, 8 females, mean age 56±3,8 years). Lipid profile, C-reactive protein, fibrinogen, carotid artery structural and hemodynamical changes and state of endothelial function of brachial artery were studied before and after 12-weeks of treatement. In 29 patients with significant disorders investigations of carotid arteries and vessel endothelial function were prolonged up to 6 month. During 12-weeks study period Vasilip lowered TC, LDL-C, TG and AR by 27%,37%, 16% and 39% respectively. Flow-mediated endothelium – dependent vasodilatation (FMD) impared at baseline (5,3±0,9%) improved up significantly and became 9,1±0,34% after 12 weeks. During following weeks FMD was increasing gradually and became 9,5±0,77% after 6 months therapy ($p<0,001$). Enhanced thickness of carotid arteries intima-media complex :1,3±0,21mm decreased and became 1,13±0,78mm and 11±0,36mm after 12 weeks and 6months respectively ($p<0,01$). During this period we observed remodelling-stabilization of atherosclerotic plaques in 13 patients. Carotid arteries occlusion degree reduced from 37,5±3,4% to 22,5±2,3%. Compared with baseline all reductions were statistically significant at the end of 6 months Vasilip treatment ($p<0,001$). So, patients with CHD before statin treatment had impaired endothelial function , that was improved significantky after Vasilip treatment. By examination of plasma C-reactive protein and fibrinogen concentrations there was observed one more pleiotropic effect of simvastatin – anti-inflammatory action. At the end of supervised period - after 12 weeks of therapy mean plasma levels of C-reactive protein were 1,9±0,76 mg/Dl. Compared with baseline (3,8±0,65 mg/dL) reduction was statistically significant ($p<0,001$). Mean plasma fibrinogen concentration before statin treatment was 6,9±1,1g/L and at the end of the supervision it was 3,2±0,4g/L($p<0,01$). Thus, Vasilip (20mg) daily improved lipid spectrum and revealed beneficial effects beyond cholesterol lowering, the so-called pleiotropic effects: it significantly improved endothelial function and carotid arteries lesion, revealed anti-inflammatory activity. These lipid and non-lipid effects that were more significant during long-term therapy are very important for the prevention of reccurent ischemic events.

## EFFECTS OF ROSUVASTATIN ON FASTING AND POSTPRANDIAL LIPOPROTEIN AND FATTY ACID METABOLISM IN COMBINED HYPERLIPIDEMIC MALE PATIENTS WITH PREMATURE CORONARY SCLEROSIS

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Postprandial hyperlipidemia is associated with premature coronary artery disease (CAD) and can improve by statins. We investigated the effects of rosuvastatin on postprandial lipoprotein and fatty acid metabolism in 20 male premature CAD patients (50±4 years). Self-determined daylong capillary triglycerides (TGc) were measured off-treatment and after rosuvastatin 20 and 40mg/d. Standardized oral fat-loading tests (OFLT) were performed off-treatment and after 40mg/d. Twenty age- and waist-matched healthy controls served as a reference group. Rosuvastatin 20mg/d improved fasting LDL-cholesterol, plasma TG, apolipoprotein B and HDL-cholesterol (-52%, -37%, -37% and +25%, $P<0.01$ each). Total daylong triglyceridemia (area under the TGc-curve, TGc-AUC) was reduced by 33% ($P<0.001$), but remained higher than in the reference group. Rosuvastatin 40mg/d did not show significant additional effects on fasting lipids and daylong TGc. Tested by OFLT, rosuvastatin 40mg/d reduced plasma TG-AUC by 23% ($P<0.005$), reaching reference values. Whereas strong and significant reductions in the fasting and postprandial cholesterol content of chylomicron, VLDL1 and VLDL2 fractions were achieved, the TG reduction in these lipoproteins was less pronounced. Furthermore, rosuvastatin normalized postprandial complement component 3 and hydroxybutyric acid changes, indicative of improved peripheral fatty acid (FA) trapping and reduced hepatic FA flux. In conclusion, in combined hyperlipidemic patients with premature CAD, rosuvastatin improves postprandial fatty acid and lipoprotein metabolism, the latter by strongly reducing the cholesterol content of triglyceride-rich lipoproteins. These pleiotropic effects of rosuvastatin contribute to CAD risk reduction.

## LIPID RESPONSES TO EZETIMIBE IN CLINICAL PRACTICE

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Ezetimibe is the first member of a new class of lipid lowering drug which inhibits intestinal absorption of dietary and biliary cholesterol.
I have evaluated its use in lipid clinic patients in whom lipids were not optimally controlled by a previous healthy lifestyle and maximally tolerated medication. Fasting lipid profiles were examined in 100 patients before and after at least 4 weeks treatment with Ezetimibe. In 85 of them Ezetimibe was added to existing therapy – 20 on no lipid lowering medication, 54 on statins and 11 on fibrates. In the remaining 15, who were on a statin plus fibrate, statin therapy was continued and the fibrate replaced by Ezetimibe.
In the 85 patients where Ezetimibe was added the mean changes were total cholesterol (TC) -18%, triglyceride (TG) -14%, HDL cholesterol (HDLC) + 2% and LDL cholesterol (LDLC) -24%. LDLC changes ranged from +7 to -54%. In monotherapy use mean changes were TC -14%, TG -7%, HDLC 0% and LDLC -21% (range -9 to -33%). When added to a statin mean changes were TC -21%, TG -20%, HDLC -1% and LDLC -28% (range +7 to - 54%). When added to a fibrate mean changes were TC -15%, TG -13%, HDLC +3% and LDLC -20% (range -9 to -27%). In the 15 patients on statin plus fibrate, where the fibrate was replaced by Ezetimibe, mean changes were TC -24%, TG -3%, HDLC -6% and LDLC -37% (range -10 to -68%).
The effect of Ezetimibe on all components of the lipid profile in this group of patients is encouraging for both monotherapy and in combination with statins or fibrates. The range of LDLC response in monotherapy is likely to relate, at least in part, to the between-person variability in cholesterol absorption, the even bigger range in statin treated patients to both this and differences in cholesterol synthesis. The addition of Ezetimibe to fibrates is not currently licensed but may be useful where fibrates are the drug of choice or where fibrates are tolerated but statins are not. In patients with low HDLC and / or raised TG the further improvements seen in this situation may be particularly useful. Where a fibrate has been added to a statin with the main aim of lowering LDLC, the replacement of the fibrate by Ezetimibe can produce a significant benefit with, on average, a 37% further reduction in LDLC.

## ACHIEVEMENT OF APOLIPOPROTEIN B GOAL IN PATIENTS WHO ACHIEVE THEIR LOW-DENSITY LIPOPROTEIN CHOLESTEROL GOAL: RESULTS OF THE MERCURY I TRIAL

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Apolipoprotein B (apo B) is the major apolipoprotein in all atherogenic lipoproteins. A total apo B goal has been recommended as a secondary target after the appropriate low-density lipoprotein cholesterol (LDL-C) goal has been met. This multinational trial (4522IL/0081) assessed the efficacy of rosuvastatin to bring hypercholesterolemic pts with CHD, atherosclerosis or type 2 diabetes and fasting LDL-C ≥2.99 mmol/L (≥115 mg/dL) to these primary and secondary endpoints when switched from atorvastatin, simvastatin or pravastatin. After a 6-wk dietary lead-in, 3140 adults were randomized to open-label rosuvastatin 10 mg (R10), atorvastatin 10 mg (A10), atorvastatin 20 mg (A20), simvastatin 20 mg (S20) or pravastatin 40 mg (P40) for 8 wks. Pts then stayed on these treatments or switched for 8 more wks from A10, S20 and P40 to R10 or from A20 to R10 or rosuvastatin 20 mg (R20). Table shows wk-16 comparisons of % pts achieving LDL-C and apo B goals (all risk categories combined) using logistic regression analyses.

| Period 1 (wks 0–8) | Period 2 (wks 9–16) | % achieving LDL-C goal[1] at wk 16 | % achieving LDL-C[1] + apo B[2] goals at wk 16 |
|---|---|---|---|
| R10 (n=538) | R10 (n=521) | 79 | 50 |
| A10 (n=529) | A10 (n=240) | 69 | 34 |
| | R10 (n=276) | 79* | 49* |
| A20 (n=925) | A20 (n=299) | 74 | 46 |
| | R10 (n=293) | 78 | 50 |
| | R20 (n=305) | 86* | 62* |
| S20 (n=543) | S20 (n=250) | 60 | 29 |
| | R10 (n=277) | 75* | 45* |
| P40 (n=521) | P40 (n=253) | 50 | 24 |
| | R10 (n=253) | 80* | 53* |

[1]<160 mg/dL, <130 mg/db L, <100 mg/dL for low-, medium-, and high-risk patients, respectively (Adult Treatment Panel III. JAMA 2001;285:2486-97.)
[2]<130 mg/dL, <110 mg/dL. <90 mg/dL for low-, medium-, and high-risk patients, respectively (Grundy SM. Circulation 2002;106:2526-9.)
*p<0.001, R10 vs A10, S20, or P40 & R20 vs A20

Over 16 wks, all treatments were well tolerated, with similar adverse event types and occurrence rates among treatment groups. There were no cases of myopathy. Switching to rosuvastatin from comparator statins at the doses studied brought more pts, including those at high risk of coronary artery disease, to their primary LDL-C and secondary total apo B treatment goals.

## PRAVASTATIN REDUCES PLASMA LEVELS OF OXIDIZED LOW-DENSITY LIPOPROTEIN IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Background** Oxidized phospholipids (OxPL) are present in atherosclerotic plaques and bound by lipoprotein (a) (Lp(a)) in plasma. We evaluated the effect of two-year pravastatin on oxidized low-density lipoprotein (LDL) in 179 children with familial hypercholesterolemia (FH).
**Methods and Results** Eighty-eight children were randomized to placebo and 91 children to pravastatin (20-40mg). We measured the OxPL content on apolipoprotein B-100 (ApoB) detected by antibody E06, ApoB immune complexes (IgG IC and IgM IC), OxLDL autoantibodies and Lp(a) levels. The OxLP and IC data are reported per ApoB particle (OxPL/ApoB, IC/ApoB) and as total levels on all ApoB particles (Total ApoB-OxPL and Total IC). As compared to placebo, ApoB decreased by 18.4 % ($p$ <0.001), total ApoB-OxPL by 11.9% ($p$=0.008), total ApoB-IC IgG by 10.3% ($p$=0.063), and IgM by 28.7% ($p$ <0.001). When normalized per ApoB, pravastatin increased OxPL/ApoB by 9.5% ($p$=0.016), IgG IC/ApoB by 11.9% ($p$=0.043) and Lp(a) by 11% ($p$=0.77) as compared to placebo. Furthermore, there was a strong correlation between OxPL/apoB and Lp(a) at baseline ($r$=0.845 $p$<0.001).
**Conclusion** Pravastatin reduced total OxPL on all ApoB particles in children with FH. However, there was an enrichment of OxPL on a smaller pool of ApoB particles in parallel with an increase in Lp(a). This suggests that OxPL is mobilized and bound by Lp(a), and that pravastatin promotes the reduction, mobilization and clearance of OxPL from the vessel wall and circulation. This may contribute to the prevention of premature atherosclerosis in children with FH.

## EFFECT OF PRAVASTATIN ON L0W-DENSITY LIPOPROTEIN PARTICLE CONCENTRATIONS IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Previous studies demonstrated that both elevated levels of LDL particles as well as small dense LDL might be associated with cardiovascular disease (CVD). We examined LDL subclasses and particle concentrations by nuclear magnetic resonance (NMR) spectroscopy in 143 children (8-18 years) with familial hypercholesterolemia (FH) and their healthy siblings (n=48). We also evaluated the effect of 1-year pravastatin (20-40mg) on LDL subclasses and particle concentrations in these children.
FH children had significantly higher concentrations of small dense LDL as compared to controls (18.7 ± 18.0 mg/dL vs 6.2 ± 8.4 mg/dL; $p<0.001$), higher intermediate size LDL concentrations (30.8 ± 32.8 mg/dL vs 22.3 ± 17.8 mg/dL; $p=0.025$), and higher large size LDL concentrations (117.7 ± 42.9 mg/dL vs 54.4 ± 23.8 mg/dL; $p<0.001$). Mean LDL particle concentration was also higher than in controls (1698 ± 354 nmol/L vs 840 ± 179 nmol/L; $p<0.001$). As compared to placebo, 1-year pravastatin reduced LDL particle concentrations by 345 ± 47 nmol/L (19.7%), but small dense LDL particles did not decrease significantly.
In conclusion, children with FH have a higher concentration of small dense, intermediate and larger LDL as well as higher levels of LDL particles per se when compared to controls. Although the concentration of small dense LDL was increased, no significant effect was observed of pravastatin therapy, indicating the need fore more aggressive lipid lowering in these FH children.

## EFFECTS OF STATINS ON LIPID AND LIPOPROTEIN PROFILE IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Objective:
Clinical research of the recent decade have shown high efficacy of statins in FH therapy. Pediatric recommendations include first step diet and bile acid-binding resins. Hover, such therapy is not always effective. The purpose of this study is to evaluate the effects of statins on lipid and lipoprotein profile and FH clinical picture.
Methods: The study covered 35 FH children, divided into two groups. The first group, comprising 25 children (15F and 11M), mean age 13 years and 10 months, was administered 0.4 mg/kg/d simvastatin for 6.5 months.
The other group, i.e. 10 children (5F, 5M), mean age 14 years, was administered 0.32 mg/kg/d atorvastatin for 8 months. Plasma lipid, lipoprotein LDL-cholesterol, alanine aminotransferase and creatine kinase were measured every 6 months. In a boy aged 16 years arterial hypertension was found, and in another, 14-year old boy, Doppler test of cervical arteries revealed basement membrane lesions.
The children were administered step one diet for 3-11 months prior to and during statins therapy.
Results: In both groups simvastatin and atorvastatin caused significantly high reduction of plasma TC-30.2%, -25.8%, L lipoprotein LDL - C 36.2%, -28.8%, and TC/HDL atherogenic index of -28.9%, -24.0%, respectively. The groups showed no differences in post-therapy lipid and lipoprotein concentration, and no correlation was found between statins dose per kg/d and lipid and lipoprotein profile changes.
In group one simvastatin therapy resulted in diastolic pressure reduction ($p<0.05$).
Following 10 months' simvastatin therapy, 14-year-old boy showed no endothelium changes.

## PRAVASTATIN IMPROVES POSTPRANDIAL ENDOTHELIAL DYFUNCTION IN PATIENTS WITH STABLE EFFORT ANGINA PECTORIS

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Postprandial hyperlipidemia and hyperglycemia as well as hemorheological deterioration have been reported to be atherogenic. We examined the short-term effects of pravastatin on endothelial function and hemorheological nature before and after a test meal in patients with stable effort angina pectoris (AP). Twenty five AP patients (62±13 yrs) who met criteria of FPG<126, TG<250, LDL-C< 200 (mg/dl) and HbA1c <7.0%, and 6 normal control subjects (53±8 yrs) were studied. We measured forearm blood flow (FBF) by strain-gauge plethysmography during reactive hyperemia, whole blood transit time (WBTT) using a microchannel array flow analyzer, and plasma levels of glucose, insulin and lipids before and 2 hrs after a test meal (680 KCal, 40g fat). All the measurement protocol repeated two days after administration of pravastatin (20mg/day). In AP group, LDL-C and TG were significantly higher than control both before and after meal, while glucose and insulin higher only after meal. FBF in AP group was significantly lower than control both before (24.1±8.4 vs. 36.7±4.7 ml/min per 100ml tissue, $p<0.05$) and after meal (23.8±7.6 vs. 39.2±3.3, $p<0.01$). WBTT showed no significant difference between the two groups. Two-day pravastatin treatment reduced TC, and LDL-C levels both before and after meal, and increased postprandial FBF in AP patients (23.8±7.6 to 28.1±6.3, $p<0.05$), but did not change WBTT. Endothelial dysfunction, especially that after meal was observed in AP patients, which pravastatin restored even after only 2 days administration.

## FIBRATES THERAPY MODULATE INFLAMMATORY ACTIVATION IN HYPOALPHALIPOPROTEINEMIA.

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**Objectives.** Anti-inflammatory properties of HDL, could account for the protective effect of HDL against atherogenesis. We have recently shown that Familial Hypoalphalipoproteinemia, (Hypoalpha) is characterized by inflammatory activation, being a model to study the relation between lipid variations and inflammation. We studied the effect of a short time fenofibrate therapy on lipid profile as well as on inflammation markers in Hypoalpha subjects.
**Methods and Results.** In Patients (n 22) affected solely by Hypoalpha, a complete lipid profile and inflammation markers (C-reactive protein, complement proteins, adhesion molecules, ceruloplasmine, haptoglobine, antitrypsin, α-1 acid glycoprotein) were determined. Patients were divided into two groups: group 1 (n. 11), placed on fenofibrate therapy (8 men, mean age 52±10) for 3 months, and group 2 (n.11), served as control without pharmacological therapy (9 men, mean age 51±13).
We have observed that therapy with fibrates not only shown to improve the lipid profile in group 1 (basal *vs* 3 month therapy: HDL 35.5±5.6 *vs* 39.0±5.0 $p<0.0005$; ApoA-I 112.8±13.4, *vs* 120±13.6 $p<0.05$, ApoA-II 30.4±2.4 *vs* 32.2±4.3 $p<0.05$, TG 207.1±147.3 *vs* 134.9±68.3 $p<0.05$), but also to induce a meaningful reduction of C-reactive protein (median 0.25, range 0.03-1.35 *vs* 0.11, 0.07-0.34; $p<0.01$), proteins complement (C3 170.4±43.0 *vs* 143.3±27.6, $p<0.001$; C4 42.8±27.0 *vs* 26.6±9.1, $p<0.05$), haptoglobine (144.1±51.4 *vs* 112.3±31.9, $p<0.05$) and α-1 acid glycoprotein (90.0±27.2 *vs* 76.5±19.1, $p<0.05$).
**Conclusions.** These preliminary results indicate that the raise of HDL levels and improvement in lipid profile by fenofibrate therapy can modulate the inflammatory state observed in Hypoalpha patients.

## IMMUNE MODULATION EFFECT OF ATORVASTATIN IN PRIMARY HYPERCHOLESTEROLEMIA.

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**Objectives.** Atherosclerosis is currently considered "an inflammatory disease", as atherosclerotic lesion, in its different development phases is characterized by inflammatory/immune system activation. Whereas Hypercholesterolemia is an established model of atherosclerosis, we studied whether Primary Hypercholesterolemia (PHC), with or without clinical atherosclerosis, is associated with high values of immune system markers and whether they are sensitive to atorvastatin therapy.

**Methods and Results.** We examined the levels of sICAM-1, C3, C4 complement fractions in 48 patients with PHC, with (CAD group) or without (No CAD group) coronary artery disease (CAD) in comparison to a group of 48 healthy controls. The two groups of patients were studied before and after atorvastatin therapy.

Both hypercholesterolemic groups, compared to controls, showed higher mean values of sICAM-1, C3 and C4 ($p<0.0001$). The two groups of patients responded differently to atorvastatin therapy. After 3 months C3 normalised in both patients groups ($p<0.001$ with respect to basal values); C4 was greatly reduced only in CAD group ($p<0.01$). After 12 months the effect of therapy on C3 persisted; in the CAD group C4 values showed a further reduction ($p<0.05$ with respect to three months therapy); after 12 months sICAM-1 values showed significant reduction ($p<0.001$ with respect to basal values) only in the CAD group.

**Conclusions.** High plasma values of C3 and C4 in PHC cluster with high values of sICAM-1, discriminate subjects with coronary artery disease and could be used to monitor the anti-inflammatory effect of statins in these patients.

## PRAVASTATIN IMPROVES INSULIN RESISTANCE IN HYPERLIDEMIC PATIENTS

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Insulin resistance is a metabolic syndrome associated with hypertension, hyperlipidemia, and impaired glucose tolerance. Therefore, it is important to know whether or not the drugs used to treat these diseases have a favorable or unfavorable effect on insulin resistance. Research into the effect of statins on insulin resistance is inconclusive. This study used a meal test that consisted of 115g of cookies (energy 560kcal; glucose 75g; protein 7g; fat 24g) to evaluate the effect of statins on insulin resistance in hypercholesterolemic patients.

Fifty-five hypercholesterolemic patients (TC 220mg/dl) treated pravastatin at 10mg/day were tested by fasting for 12hr, after which the meal test was orally administered. Venous blood samples were collected before and one and two hours after eating the meal. Plasma glucose (PG), IRI, total cholesterol (TC), HDL-C, and apolipoprotein B levels were measured. After one year of follow-up, 25 patients who continued to take pravastatin were re-tested. Insulin resistance was assessed by the area under the IRI curve (AUCIRI), and AUCIRI $\times$AUCG ( AUC of glucose).

The TC and apolipoprotein B levels were significantly decreased and the HDL-C level significantly increased at every sampling point of the meal test administered after one year. The PG level at 1hr was significantly decreased, and IRI at 1hr was also significantly decreased. AUCIRI and AUCIRI $\times$ AUCG were significantly decreased at the one year follow-up.

This study shows that pravastatin not only reduced serum TC, but also improved insulin resistance in hyperlipidemic patients.

## A COMPARATIVE STUDY OF STATINS AND PROBUCOL ON COMMON CAROTID INTIMA-MEDIA THICKNESS IN PATIENTS WITH MODERATE HYPERCHOLESTEROLEMIA

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To evaluate the comparative effects of various statins and probucol on the progression of carotid intima-media thickness (IMT), 179 hypercholesterolemic patients (mean age 61.2 years) were assigned to receive either simvastatin (5 mg/day, n=49), fluvastatin (20 mg/day, n=34), atorvastatin (10 mg/day, n=24), or probucol (500 mg/day, n=72). Carotid IMT was determined by B-mode ultrasound before and after one year of treatment. At baseline, no significant difference in IMT or lipids was found between the treatment groups. After 12 months of follow-up, TC, LDL-C, and IMT was reduced significantly in all groups (simvastatin, TC -18.3%, LDL-C -29.4%, IMT -13.5%; fluvastatin, TC -15.5%, LDL-C -23.8%, IMT -13.5%; atorvastatin, TC -21.4%, LDL-C -30.1%, IMT -10.0%; probucol, TC -17.3%, LDL-C -11.3%, IMT -12.6%). The reduction of LDL-C was significantly greater in the statin groups than in the probucol group, but the reduction of IMT was not significantly different between the four groups. Our data showed that simvastatin, fluvastatin, atorvastatin, and probucol all reduce carotid IMT in patients with moderate hypercholesterolemia, with no significant between group differences.

## THE EFFECT OF STATIN TREATMENT ON ENDOTHELIAL FUNCTIONS, PARAOXANASE-1 ACTIVITY, AND OXIDATIVE MARKERS OF LDL IN PATIENTS WITH HYPERLIPIDEMIA

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Serum paraoxonase(PON1) is a HDL associated enzyme that is responsible for the protective effect of HDL against LDL oxidation. The aim of this study was to determine whether statins with proven beneficial effects aganist atherosclerosis, have any effect on PON1 activity and oxidative markers of LDL with regard to the endothelial functios. **Methods**; Study population consisted of prospectively enrolled 36 patients with hypercholesterolemia. Half of them were radomizedto atorvastatin(20 mg/day) for 3 months. Endothelial functions (FMD-brachial ultrasonography), Serum PON1, and oxidative markers of LDL were determined before and after the 3 months treatment.

**Results;** After treatment, LDL levels decreased significantly, meanwhile FMD improved significantly in statin group. PON1 activity increased sligthly in statin group, however, there were no difference between the groups PON 1 activities after treatment (table). Oxidative markers (basal and stimulated MDA, basal and stimulated diens) also did not differ between the groups both before and after treatment. Also, there were no correlation between the changes observed in PON1 activity and FMD in both groups.

**Conclusion:** In this pilot study, the changes observed in PON1 activity and LDL oxidative markers did not differ between the control subjects and statin group. Any effect of statins on PON1 activity does not seem to have any contribution to their beneficial effects on endothelium.

| Comparison of groups | | | |
|---|---|---|---|
| % changes after treatment | Controls(n=14) | Atorvastatin(n=16) | p |
| Serum PON | 6±55 | 41±59 | 0.099 |
| LDL (mg/dl) | -8±21 | -40±27 | 0.003 |
| HDL(mg/dl) | -14±14 | -7±25 | NS |
| FMD(%) | 7±107 | 149±108 | 0,001 |

**STATINS INFLUENCE ON THE DINAMICS OF LIPIDS EXCHANGE PARAMETRS AND ATHEROSCLEROSIS AGGRAVATION MARKERS IN HELICOBACTERY PYLORI AND CHLAMIDIA PNEUMONIA INFECTED CHD PATIENTS**

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The aim of this work is to study the dinamics of lipids exchange parametrs and atherosclerosis aggravation markers (Fibrinogen, Protein "C" ) in Helicobactery Pylori (HP) and Chlamidia Pneumonia (CP) infected CHD patients afther statins and eradication treatment .

**Materials and Methods:** we examined CHD patients, among them: 46 with HP(confirmed by serologic diagnostic, histologicaly and by urease test) and 32 CP seropositive (more, than 1:60) patients.

For each patients Total Cholesterol (TC), Tryglicerides (TG), High Density Lipoproteins Cholesterol (HDL-C), Low Density Lipoproteins Cholesterol (LDL Cholesterol) -using Spectophotometre, Fibrinogen in plasma (by Rutbergh method)and Protein "C" befor and afther 2 months therapy were performed. HP infected patients by accepted triple thrapy (7 days) and CP infected patients by Azytromicine were treated. Among them 22 HP patients and 18 CP patients 2 months atorvastatine were received.

**Results** of investigation show that infected patients afther 2 months atorvastatine therapy had amelioration of clinical symptoms and lipid exchange parameters, than patients with only afther eradicational therapy. The inflamatory markers differed confidancly too.

**Conclusion:** aggravation of atherosclerosis greatly depends of infection. The infected CHD patients will be treated with statins (atorvastatine) under eradicational therapy.

**INTERLEUKIN-8 PRODUCTION BY POLYMORPHONUCLEAR LEUKOCYTES IN HIGH-RISK PATIENTS: CHANGES AFTER TREATMENT WITH SIMVASTATIN.**

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The aim of this study was to investigate the production of the proinflammatory chemokine interleukin (IL)-8 in polymorphonuclear leukocytes (PMNs) obtained from high-risk patients and the effects of treatment with simvastatin 20 mg/die. Patients were studied before institution of statin treatment (1D-e) and thereafter, at 3 days (3D-e) and at 30 days of treatment (30D-e). Age- and sex-matched healthy subjects were included as controls.

Eight high-risk patients (mean age 61±8 years; 5 patients with type-2 diabetes in diet treatment, 3 dyslipidemic patients; non-smokers, no heavy sporting activities) were studied. Total cholesterol, LDL-c and ApoB were found significantly reduced with respect to pretreatment values at both 3D-e and 30D-e. Both resting levels as well as fMLP-stimulated production of IL-8 in PMNs from patients at 1D-e were significantly higher than those in cells from controls (resting: 929.4±327.1 pg/ml vs 110.9±34.4; fMLP-stimulated: 1415.0±301.4 pg/ml vs 427.1±89.7 pg/ml; in both cases, $P<0.05$ by ANOVA followed by Dunnett's multiple comparison test). IL-8 production tended to decrease at 3D-e (resting: 625.5±229.8 pg/ml; fMLP-stimulated: 869.2±181.1 pg/ml) and decreased significantly at 30D-e (resting: 377.7±101.5 pg/ml; fMLP-stimulated: 687.3±74.8 pg/ml; in both cases, $P<0.05$ vs 1D-e).

In conclusion, PMNs from high-risk patients produce significantly more IL-8 than those from healthy subjects, a finding which supports the existence of an inflammatory state in these patients. Treatment with simvastatin was however associated with a time-dependent reduction of IL-8 production, which reached the values of control subjects at 30D-e. This effect may be accounted for by the concurrent reduction in both T-c, LDL-c and ApoB observed and/or by a specific anti-inflammatory effect of this statin.

**THE EFFECT OF ATORVASTATIN ON OX-LDL AND HS-CRP IN HEALTHY HIGH-RISK SUBJECTS**

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**Background and Aims:** Ox-LDL was associated with both subclinical atherosclerosis and inflammatory variables, supporting the concept that oxidatively modified LDL may play a major role in atherosclerosis development. The aim of this study was to evaluate the effect of atorvastatin on ox-LDL and Hs-CRP in healthy high-risk subjects.

**Methods:** Investigation was performed in 52 apparently healthy (< 55 y, 28 male/24 female) but high-risk subjects for premature coronary heart disease (parental myocardial infarction, increased total-C, LDL-C and Hs-CRP levels). Triglycerides, total-C and HDL-C were measured by enzymatic methods. LDL-C was calculated using the Friedewald formula. Ox-LDL was measured by a commercially available sandwich ELISA (Mercodia AB, Uppsala, Sweden). Hs-CRP was measured by an ELISA with human anti-CRP. After a baseline dietary period all subjects received atorvastatin (10 mg once daily) during six months.

**Results:** Atorvastatin significantly reduced ox-LDL (102.37±31.78 to 76.59±24.76 IU/L;$p<0.01$), total-C (6.81±0.70 to 5.05±0.60 mmol/l; $p<0.001$), LDL-C (4.77±0.74 to 3.30±0.54 mmol/l), LDL/HDL ratio (4.7±1.70 to 2.80±0.81; $p<0.001$), Tg (2.22±1.06 to 1.12±0.44 mmol/l;$p<0.006$) and Hs-CRP levels (5.91±2.41 to 2.73±1.56 mg/ml). Before statin treatment there were strong positive correlations between hs-CRP and ox-LDL ($r=0.745$;$p=0.005$), total-C ($r=0.847$;$p=0.001$) and LDL-C ($r=0.508$;$p=0.03$). After statin treatment there were significant positive correlations between ox-LDL and LDL/HDL ratio ($r=0.531$;$p=0.02$).

**Conclusion:** Early intervention with atorvastatin in healthy high-risk subjects should be prevent the development of atherosclerosis.

**CORRELATION BETWEEN C-REACTIVE PROTEIN AND BLOOD LIPIDS UNDER PROLONGED LIPRIMAR ADMINISTRATION**

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128 patients with hypercholesterolemia( ≥6.8 mmol/l) were treated with Liprimar (Atorvastatin) in a daily dose of 20 mg during 1 year. Along with the lipid spectre( Tchol, HDL, LDL, Tg, Apo-A-1 and Apo-B) CRP has been defined as well. Carotid arteries were studied by duplex scanning estimating the thickness of intima-media layer.

The investigation was carreid out before and after one-year of the mentioned hypolipidemic therapy.

The patients were divided into 2 groups depending on the level of CRP. GroupI (N=53) CRP ≥6mg/l, and groupII (N=75) CRP≤6 mg/l. In these groups the initial parameters for the intima-media thickness of carotid arteries were 1.2±0.34 mm and 1.12±0.2 mm, respectively.

The analysis of th results obtained has shown that during one year Liprimar treatment the normalization of Tchol was observed in 96% of the second group patients,while in rhe first group Tchol was normalized only in 52%.

It shold be noted as well that intima-media thickness was stabilazed against one-year Liprimar treatment i.e. became 1.23±0.42 mm(groupI ) and 1.16 ± ( groupII).

**SIMVASTATIN TREATMENT REDUCES SERUM LEVELS OF sICAM IN HYPERCHOLESTEROLEMIC PATIENTS**

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Plasma level of soluble inter-cellular adhesion molecule 1 (sICAM-1) has been proposed to be an important clinical marker for atherosclerotic associated disease. Proinflammatory cytokines and peptides like Interleukine- (IL-) 6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and vascular endothelial growth factor (VEGF) increased in acute and chronic inflammatory conditions. In this study, we compared the serum level of sICAM in hypercholesterolemic patients (HcP) with those of healthy donors. Furthermore we investigated changes of the serum level of sICAM under statin treatment. 107 HcP were treated with 20 (n=52) or 40mg (n=55) of simvastatin daily. Blood samples were taken by vein puncture at various time points. The serum concentration of soluble parameter have been measured by ELISA. Baseline levels of sICAM were significantly higher in HcP than in healthy participants (baseline levels: 351,0 ng/ml in the 20 mg group and 391,5 ng/ml in the 40 mg group *vs.* 190,0 ng/ml). 6 weeks treatment with simvastatin resulted in a significant reduction of serum level of sICAM in both groups of patients. We revealed significant higher influence of simvastatin on sICAM levels (-37,8% after 6 weeks and -55,9% after 6 months), compared to total serum cholesterol and LDL. There were no significant correlation between the serum concentration of sICAM with other proinflammatory peptides measured in our study (all P>0,05). Statins reduced sICAM apart from cholesterol lowering. This novel anti-inflammatory effect of statins might partly contribute to the clinical benefits of these drugs.

**ONE YEAR ATORVASTATIN TREATMENT IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA : EFFECTS ON CRP AND FIBRINOGEN**

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Statins are the drugs of choice in heterozygous Familial Hypercholesterolemia (FH), which has a high risk of premature cardiovascular events. A one year open-label study was conducted to test efficacy and tollerability of atorvastatin titrated to the target, in proven FH patients evaluating some inflammatory parameters. One hundred and two FH patients (44 men and 58 women; mean age 58.7 ± 3.6 years) were included in the study. After evaluation using B-mode Duplex scanning system of extracranial carotid arteries, patients were separated into two groups: Group 1 (n. 40 -15 men, 25 women) with carotid plaques or intima-media thickness; and Group 2 (n. 62 - 30 men, 33 women) without carotid lesions. All patients were submitted to atorvastatin treatment titrated to achieve the therapeutic target (LDL belowe 100 mg/dl). Patients with carotid lesions were also submitted to oral fixed dose of ASA 100 mg/daily. In both group of patients without and with carotid lesions, Atorvastatin treatment, at the mean dosage of 23.5 mg./daily, reduces Triglycerides of 8.7% ($p< 0.005$) and 10.6% ($p<0.005$) , Total Cholesterol of 41.5% ($p< 0.005$) and 42.6% ($p< 0.005$), LDL–C of 55.8% ($p< 0.005$) and 57.3% ($p < 0.005$), APO B of 38.3% ($p < 0.005$) and 37.2% ($p< 0.005$) respectively , and increases the mean levels of HDL–C of 8.7% ($p< 0.005$) and 11.0% ($p< 0.005$), APO AI of 3.2% ($p< 0.05$) and 3.3% respectively. In both group of patients the mean decrease (52 weeks) of fibrinogen was of 19.8% ($p< 0.005$) and 10.4% ($p < 0.005$) respectively and of hs-CRP of 36.2% ($p < 0.005$) and 38.2% ($p< 0.005$) respectively. No variation of the parameters of safety and clinical tolerability of the drugs administrated were observed. In FH patients, one year Atorvastatin treatment titrated to the target at mean dosage of 23.5 mg./daily shows a powerful efficacy with a good tollerability and improves the outcame of these patients by a persistent reduction of serum lipid levels and inflammatory parameters.

**THE REGRESSION BETWEEN MULTIPLE INFLAMMATORY MARKERS AND C-IMT IN NORMOCHOLESTEROLEMIC STABLE IHD. THE EFFECT OF MODERATE DOSE OF ATORVASTATIN (MIAMI STUDY)**

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Introduction: Plasma levels of *Hs-CRP* may be useful to select subjects at high risk of cardiovascular events. This inflammatory marker also helps identify patients most likely to respond to statins.
Aim: A prospective clinical study was designed to investigate in a population of stable IHD patients treated with Atorvastatin:

a) the regression of CRP levels and other soluble inflammatory markers;
b) the correlations between levels of Carotid Intima Media Thickness (C-IMT) and a series of soluble markers at different times;
c) the influence of Hs-CRP levels on C-IMT regression;
d) the utility of C-IMT as an additional marker to identify patients likely to respond to statins.

Materials and Methods: MIAMI is an open, multicenter, independent study. In 100 patients with previous MI (> two months), in stable clinical conditions, with normal cholesterol and blood glucose, receiving atorvastatin (20 mg/daily) for 24 months, the following variables were measured at 0, 12 and 24 month: VCAM, ICAM, E-selectins, IL-6, -8, -10, -18, TNF-alpha, hs-CRP, MMP-9, TF, TFPI, Fg, TC, HDL, LDL, TG, urinary isoprostanes. C-IMT was measured blindly by computerised determination of images recorded in three examinations.
The study started in January 2003 and is scheduled to end in December 2005.

Topic Preferences:

1) New Markers for Cardiovascular Risk (18.)
2) Inflammation and Atherosclerosis (21.)
3) Pleiotropic effects of Lipid Lowering Drugs (41.)

**COMPARATIVE ESTIMATION OF EFFICACY AND SAFETY OF EARLY TREATMENT WITH STATINS AT PATIENTS WITH Q WAVE MYOCARDIAL INFARCTION**

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**Background.** Despite of favorable results of early treatment with statins in patients with ACS without ST elevation efficiency, expediency, terms of the beginning and a regimen of statins dosing in patients with Q wave MI remain insufficiently investigated. We put the purpose to study interrelation of lipid and nonlipid mechanisms of action and clinical efficiency of simvastatin in usual therapeutic doses independently from the baseline cholesterol level in patients with Q wave MI.
**Methods and results.** 79 patients with acute Q wave MI were examined (77% men). 23 patients composed a control group and 56 receiving in addition to standard basic therapy (aspirin, beta-blockers, ACE inhibitors) from the first 24 hours of admission simvastatin (Zocor) in daily dosage 20 mg within 3 months. Groups were comparable on frequency of thrombolytic therapy, to character and doses of basic medications, sex, age, presence of CHD before MI, hypertension and MI location. Additional researches included a standard lipidogram, an estimation of systolic (LVEF) and diastolic functions of LV, determine of basic classes Ig levels, circulating immune complexes (CIC) and superoxyde anion production (spontaneous and induced with the atherogenous CIC separately in neutrophiles and monocytes populations. Reliably ($p<0.05$) lower frequency of CHF (Killip III-IV), re-MI, number of ischemic episodes demanding nitrate using were registered in simvastatin early treatment group. The minimal expressiveness of remodeling processes (according to LV end diastolic volume, LVEF) and amelioration of parameters of diastolic function (E/A) alongside with normalization of superoxyde anion production with improvement of functional reserves of both phagocytes populations also were observed in the patients treated with simvastatin. There was no strong association between positive lipid and nonlipid effects of statin treatment.
**Conclusions.** Thus, early application of statins (from the first hours) in Q wave MI is effective and safe. Efficiency of therapy is caused more by nonlipid and also lipid mechanisms. The significant effect is achieved by using of therapeutic doses of simvastatin (Zocor).

## NOVEL PLEIOTROPIC EFFECT OF PITAVASTATIN ON THE MIGRATION OF VASCULAR SMOOTH MUSCLE CELLS

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Pitavastatin is a recently developed potent inhibitor of HMG-CoA reductase. Statins are known to show various actions, which are known as pleiotropic effects on vascular cells including the modulation of proliferation of vascular smooth muscle cells (SMCs). We have identified that LR11, an LDL receptor gene family member, is a novel regulator of SMCs migration (Zhu et al. Circulation 2002;105:1830-6). LR11 mediates urokinase-type plasminogen activator receptor (uPAR) localization on the plasma membrane, since both the membrane-spanning and the secreted forms of LR11 bind to and co-localize with uPAR on the cell surface (Zhu et al. Circ Res 2004;94:752-8). Here we show the novel mechanism of pitavastation on the modulation of migration activity of SMCs through the LR11/uPAR system. PDGF-BB stimulated the expression of LR11 in the concentration range at which it acts as a stimulant of migration of cultured SMCs. The induced expression of LR11 caused the increased cell surface localization of uPAR. Pitavastatin reduced the PDGF-BB -induced surface expression of LR11 and uPAR dose-dependently. The inhibitory effect was observed by simvastatin at lesser extent, but not pravastatin. The increased migration in LR11-overexpressing cells or in SMCs with the secreted soluble LR11 is not reversed by the incubation with pitavastatin. Thus, pitavastatin shows the novel pleoiotropic effect on the PDGF-induced migration through the LR11/uPAR system in SMCs.

## FUNCTIONAL RESPONSES OF POLYMORPHONUCLEAR LEUKOCYTES IN HIGH-RISK PATIENTS: EFFECTS OF SIMVASTATIN

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The aim of this study was to investigate whether the treatment with simvastatin 20 mg/die may change polymorphonuclear leukocyte (PMN) function in high-risk patients. To this end, the chemotactic index (CI, i.e. stimulated chemotaxis/spontaneous migration) and reactive oxygen species (ROS) production in isolated PMNs obtained from patients before institution of statin treatment (1D-e) and thereafter, at 3 days (3D-e) and at 30 days of treatment (30D-e). Functional responses were obtained by stimulation of the cells with fMLP, a chemotactic peptide acting on membrane receptors, and PMA, a direct activator of protein kinase C.

Eight high-risk subjects (mean age 61±8 years; 5 patients with type-2 diabetes in diet treatment, 3 dyslipidemic patients; non-smokers, no heavy sporting activities) were studied. In patients at 1D-e the mean total cholesterol (T-c) was 238±23 mg/dl, LDL-c was 165±17 mg/dl, HDL-c was 47.5±5.3 mg/dl, and triglycerides were 125±50 mg/dl. T-c, LDL-c and ApoB significantly decreased at both 3D-e (202±27 mg/dl, 134±25 mg/dl, and 108±15 mg/dl, respectively) and 30D-e (164±28 mg/dl, 96±21 mg/dl, and 70±32 mg/dl, respectively). Differences were always statistically significant vs 1D-e ($P<0.05$ by ANOVA followed by Student Newman Keuls post test). The dietary habits (as evaluated by a diary) and the fasting glycemia did not change during the 30-days follow-up. The CI in PMNs from patients was 1.29±0.06 at 1D-e and did not change at 3D-e and at 30D-e, while PMA-induced ROS production was significantly reduced at the 30D-e, both with respect to 1D-e and 3D-e ($P<0.05$ vs both 1D-e and 3D-e). By contrast, fMLP-induced ROS generation remained unchanged throughout the treatment.

In conclusion, the treatment with simvastatin in high-risk patients is associated with a reduction of stimulated ROS production by PMNs. The effect is stimulus specific, and this finding may support an action of simvastatin on intracellular (rather than membrane receptor) targets in the modulation of the inflammatory response.

## LIPID LOWERING DRUGS INDUCE COMPLEMENT RESISTANCE INDEPENDENT OF EXPRESSION OF COMPLEMENT REGULATORS

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Lipid lowering drugs have successfully been used to reduce the occurrence of cardiovascular events like atherosclerosis and myocardial infarction. Complement has been implicated in these events and we have investigated whether lipid lowering drugs can affect the C-susceptibility of nucleated cells. A variety of cells including human and mouse monocyte/macrophage cell lines, primary porcine vascular cells and human B cell lines were cultured in the presence of simvastatin or fenofibrate. Expression of the C-regulators MCP and CD59 was not altered while on certain human cells after culturing in the presence simvastatin a small increase in DAF was observed. On most cells an increase in resistance to pore formation and lysis by the membrane attack complex (MAC) of complement was observed after incubation with simvastatin and fenofibrate. C-mediated lysis was observed independent of the expression of DAF. Culturing cells in the presence of simvastatin or fenofibrate not only induced an increased resistance to C-mediated lysis, but also an increased resistance against lysis by the cholesterol dependent toxin streptolysin O, which has a similar lytic mechanism and lipid requirement (including cholesterol) as the MAC. In contrast, cells became more susceptible to lysis by the bee venom peptide melittin. Lysis by melittin is more efficient at lower plasma membrane cholesterol levels. These results suggest that simvastatin and fenofibrate protected the cells against C-mediated lysis by reducing the cholesterol content of the cell membrane, thereby reducing the insertion of the MAC.

In conclusion our results show that lipid lowering drugs can alter the C-susceptibility of nucleated cells, most likely due to a change in membrane composition resulting in a reduced insertion of the MAC, which may contribute to their beneficial pleiotropic effects *in vivo*.

## REDUCTION OF SERUM UBIQUINOL-10 AND UBIQUINONE-10 LEVELS BY ATORVASTATIN IN HYPERCHOLESTEROLEMIC PATIENTS

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Backgrounds: Inhibition of HMG-CoA reductase by statin results in decreased synthesis of cholesterol and other products downstream of mevalonate, which may produce adverse effects in statin therapy.

Methods: We have studied the reductions of serum ubiquinol-10 and ubiquinone-10 levels in hypercholesterolemic patients treated with atorvastatin. Fourteen patients were treated with 10 mg/day of atorvastatin, and serum lipid, ubiquinol-10 and ubiquinone-10 levels were measured before and after 8 weeks of treatment.

Results: Serum total cholesterol and LDL-cholesterol levels decreased significantly. All patients showed definite reductions of serum ubiquinol-10 and ubiquinone-10 levels, and mean levels of serum ubiquinol-10 and ubiquinone-10 levels decreased significantly from $0.81 \pm 0.21$ to $0.46 \pm 0.10$ $\mu$ g/ml ($p<0.0001$), and from $0.10 \pm 0.06$ to $0.06 \pm 0.02$ $\mu$ g/ml ($p=0.0008$), respectively. Percent reductions of ubiquinol-10 and those of total cholesterol showed a positive correlation ($r=0.627$, $p=0.0165$).

Conclusions: As atorvastatin reduces serum ubiquinol-10 as well as serum cholesterol levels in all patients, it is imperative that physicians are forewarned about the risks associated with ubiquinol-10 depletion and the need for prophylactic supplementation with ubiquinol-10 to reduce those risks.

## ANTIOX IDATIVE EFFECTS OF PITAVASTATIN TO LIPOPROTEINS.

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To clarify the pleiotropic effect of pitavastatin, perosidizability of VLDL, LDL and HDL before and after pitavastatin administration was investigated in hyperlipemic patients.

Pitavastatin (2 mg/day) was orally administered to 38 patients with hyperchole sterolemia for 4 weeks, and VLDL, LDL and HDL were isolated by ultracentrifugation to measure lipid peroxide ( LPO). LPO of each lipoprotein was estimated before drug administration and after one in 0, 6, 12 and 24 hours by dialyzing against 5 $\mu$ mol CuCl2. LPO produced hourly in each lipoprotein after dialyzing was markedly suppressed by pitavastatin administration. The suppression in LDL was greatest among 3 lipoproteins. Total cholesterol was tremendously decreased.

Conclusion.

Pitavastatin is very useful antiatherogenic drug which suppresses the production of lipid peroxide in lipoproteins and also decreases markedly serum cholesterol level.

## FLUVASTATIN PROTECTS THE FUNCTION OF ACTIVE OXYGEN-CONSUMPTION ON ARTERIAL VESSEL WALL

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Y, Yoshiki and K, Okubo reported that chemiluminesence intesty [P] brighted in the presence of active oxygen spaces [X], catalytic spaces [Y] and receptors [Z] was predicted by [P]=[X][Y][Z] ( Photochemistry and Photobiology, 73, 545-550, 2001). Previously, we reported that arterial vessel ( intima, media and adventitia) showed Y and Z functions, especially strong Z function.

So the effect of fluvastatin to Z function was investgated in this study. The aortic vessels of cholesterol (0.5%) feeding-rabbits were used (control : 9 , cholesterol feeding : 9, cholesterol feeding wich fluvastatin 1~3 mg : 23).

Z photon intensity on arterial vessel wall in control was very strong, but it was clearly suppressed by cholesterol feeding. However, Z photon intensily on artrial vessel wall recovered by fluvastatin administration.

Conclusion. Consumption mechanism of active oxygen exists on the arterial vessel wall according to XYZ chemiluminescence system. Cholesterol accumulation on the vessel wall suppresses markedly Z function, but fluvastatin protects very well the Z function.

## EXTRALIPID EFFECTS OF ATORVASTATIN IN PATIENTS WITH DYSLIPIDAEMIA IIa

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Large evidence clearly suggests multifactorial background of atherogenesis with a crucial role of inflammation process cells. Monocytes are suggested to be a key cells and play an important role in every stage of atherosclerotic plaque formation and its complications. Interleukin 1β (IL-1β) is a main proinflammatory and procoagulatory cytokine secreted by monocytes, which enhances leukocytes' adhesion and transmigration into subintimal space. Not only hypolipemic effects of Statins have been observed, but also growing evidence strongly reveals its pleiotropic effects on endothelial local inflammation and coagulation / fibrynolisis balance. We decided to investigate IL-1 β gene expression and IL-1β secretion in monocytes isolated from patients with diagnosis of dyslipidemia IIa.

Twelve individuals suffering from biochemically confirmed hyperlipoproteinemia type IIa (HLPIIa), who have failed to respond to dietary control, were treated with atorvastatin ( 20 mg/d ) for 1 month. The control group included thirteen healthy age-matched volunteers. Any health disorders including inflammatory diseases were excluded in all participants. IL-1β concentrations in cultured monocytes were measured using ELISA method. In order to evaluate the expression of IL-1β gene in monocyte the outer and nested semiquantitive RT-PCR procedures were performed. The results are normalized with the expression of Gliceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene. IL-1beta expression was not significantly higher in studied groups vs. control (outer RT-PCR: 2,19 ± 0,48 vs. 1,95 ± 0,67; nested RT-PCR: 2,24 ± 0,46 vs. 1,98 ± 0,31; $p<0,05$). After 1-month therapy with atorvastatin, significant reduction of IL-1β expression were observed (outer RT-PCR: 2,19 ± 0,48 vs. 1,14 ± 0,13; $p<0,001$, nested RT-PCR: 2,24 ± 0,46 vs. 1,66 ± 0,34; $p<0,05$). Statin treatment revealed also significant reduction of IL-1β release by cultured monocytes (133,0 ± 5,7 pg/ml vs. 77,0 ± 3,6 pg/ml; $p<0,01$). Patients suffering from atherogenic dyslipidemias reveal markedly increased IL-1β mRNA expression and monocyte release. One month treatment with atorvastatin in individuals with atherogenic dyslipidemia IIa revealed beneficial anti-inflammatory effects.

## DO STATINS HAVE AN EFFECT ON BIOCHEMICAL BONE TURNOVER MARKERS? A RANDOMIZED CONTROLLED TRIAL IN POSTMENOPAUSAL WOMEN USING ATORVASTATIN

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The use of statins has been associated with decreased risk of bone fractures in epidemiological studies. We assessed the effects of atorvastatin on biochemical parameters of bone metabolism in a multicenter, randomized, double-blind, placebo-controlled trial. Forty-nine postmenopausal women, mean age 61 ± 5 yrs., were treated with atorvastatin, 20 mg per day (n = 24) or matching placebos (n = 25) for 8 weeks. Comparing the differences to baseline between the groups, there were no statistically significant effects of atorvastatin neither on the bone formation markers intact osteocalcin and bone specific alkaline phosphatase nor on the bone resorption markers C-telopeptide and intact parathyroid hormone. The marker of bone fractures, undercarboxylated osteocalcin, was also unchanged. When analyzed in dependence of age, atorvastatin increased C-telopeptide and osteocalcin in the younger subjects while decreased them in older subjects. Interestingly, in older subjects atorvastatin caused a significant decrease in the ratio of C-telopeptide to osteocalcin, an indicator of bone remodeling, while the ratio was increased in younger subjects, suggesting beneficial effects on bone turnover exclusively in older individuals (> 63 yrs.). In summary, the present data suggest that short-term treatment with atorvastatin may have age-dependent effects on biochemical markers of bone turnover in post-menopausal women.

## USE STATINS AND RISK REDUCTION OF ATRIAL FIBRILLATION AFTER CARDIAC SURGERY

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***Objective:*** Atrial fibrillation (AF) represents the most common arrhythmic complication after cardiac surgery. The effect of preoperative use of statins on risk of postoperative AF is not known. Therefore, the aim of the current investigation was to study the effect of preoperative statin use on the risk of postoperative AF in patients undergoing cardiac surgery.
***Design and Methods:*** Patients undergoing cardiac surgery in the absence of heart failure and without significant left ventricular dysfunction (n=253, average age 65+/-11 years) were studied in a prospective fashion. Patients underwent a thorough physical examination and provided medical history. Use of cardiovascular drugs, including statins, was assessed by a standardized questionnaire or as indicated by medical records. AF was defined as episodes of more than five minutes in duration.
***Results:*** Overall, 39.1% of the total study population developed AF during the postoperative period. Surgery for valvular heart disease was associated with an about two-fold increase in the rate of postoperative AF. 57.1% of patients without, and 43.3.% of patients with postoperative AF used statins preoperatively ($p<0.05$). Patients without statin therapy had a significantly higher risk of postoperative AF compared to statin users (45.9% versus 32.8%, respectively; $p<0.05$; significance persisted after adjustment for age, heart valve surgery, and antiarrhythmic drug use). In addition, advanced age and surgery for heart valve disease increased, and use of antiarrhythmic drugs, including beta-adrenergic blockers, decreased the risk for postoperative AF by multivariate analysis ($p<0.05$).
***Conclusions:*** Use of statins significantly decreased the rate of postoperative AF in patients undergoing cardiac surgery in the absence of heart failure or significant left ventricular dysfunction. The underlying mechanism for this effect is unknown, and both, a reduction in serum cholesterol levels and cholesterol-independent pleiotropic effects, including antiinflammatory properties, of statins may be involved.

## ROSUVASTATIN REDUCED CELL DEATH THROUGH REDUCTION OF CASPASE-3 ACTIVITY AND UP-REGULATION OF ALPHA-SECRETASE IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS EXPOSED TO BETA-AMYLOID (1-42)

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**Background:** Accumulating evidence suggests that disturbances in cholesterol metabolism in the brain, as well as in the peripheral tissues, potentiate the formation and deposition of beta-amyloid (Aβ) and the progression of Alzheimer's disease (AD). Statins have been shown to decrease cholesterol synthesis through competitive inhibition of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase).
**Methods:** We have studied the effect of rosuvastatin on cell death, especially caspase-3 activity, a key effector of the caspase cascade during apoptosis in human neuroblastoma SH-SY5Y cells, and evaluated the effect of rosuvastatin on the activity of α-secretase, a key enzyme in the processing of Aβ.
**Results:** Pre-treatment of cells with rosuvastatin prior to exposure to $A\beta_{1-42}$ resulted in a significant decrease in caspase-3 activity (~53%, $p$=0.016) compared to cells treated with $A\beta_{1-42}$ alone. The reduction in caspase-3 activity was reversed by co-incubation with mevalonate (~64%, $p$=0.013). Exposure of cells to $A\beta_{1-42}$ alone or to rosuvastatin alone had little or no effect on α-secretase activity compared to control cells. Pre-treatment with rosuvastatin prior to exposure to $A\beta_{1-42}$ significantly increased α-secretase activity (~99%, $p$=0.014) compared to control cells, while co-incubation of mevalonate reversed the effect of rosuvastatin on α-secretase activity.
**Conclusion:** This is, to our knowledge, the first report that demonstrates that rosuvastatin has neuroprotective activity in human neuroblastoma SH-SY5Y cells exposed to $A\beta_{1-42}$. The protective effect is mediated through the inhibition of caspase-3 activity and up-regulation of α-secretase activity.

## PLEIOTROPIC EFFECTS OF ETOFIBRATE IN PATIENTS WITH PRIMARY HYPERTRIGLYCERIDEMIA: EFFICACY ON THE DYSMETABOLIC SYNDROME

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Hypertriglyceridemia, hyperacidemia, glucose intolerance, hyperinsulinism and insulin resistance are closely related. It is our objective to determine pleitropic effects of etofibrate through its hipolipidemic actions. Twenty-six non-diabetic patients with primary hypertriglyceridemia who maintained high triglyceride levels after at least six months of a non-pharmacological treatment, received 500 mg of etofibrate retard at dinner during 6 months. The following determinations were performed before and after the pharmacological treatment: total cholesterol and triglycerides, LDL-C, HDL-C, FFA, apo AI and apo B; and glycaemia and insulinemia during an oral glucose tolerance test. The statistical tests applied were: Chi-Square, Friedman, Nemenyi, T Test, U-Mann Whitney, Wilcoxon, and the trapezoidal rule. Using the HOMA index (cut-off point = 2,5), two groups, thirteen patients each, were obtained; nine indexes of insulin secretion and nine indexes of insulin resistance were calculated to both groups. All patients had from three to seven metabolic disturbances. After treatment, total TG, FFA and C/HDL-C ratio diminished (40%,19% and 20%, respectively), and apo AI increased (32%). Glycaemia, insulinemia, total glycemic and insulinemic areas under the curve diminished during the OGTT. One insulin sensitivity index and one insulin resistant index improved in the non-insulin resistant group; four insulin sensitivity indexes and seven insulin resistance indexes improved in the insulin resistant group (p<0,05). We conclude than after the treatment with etofibrate there was an improvement in the lipid profile with pleiotropic effects on glucose tolerance, hyperinsulinism and insulin resistance. Etofibrate is of election in the treatment of the dyslipoproteinaemia of the dysmetabolic syndrome.

## EFFECT OF LOW DOSE ATORVASTATIN VERSUS DIET-INDUCED CHOLESTEROL-LOWERING ON ATHEROSCLEROTIC LESION PROGRESSION AND INFLAMMATION IN APOE*3-LEIDEN TRANSGENIC MICE.

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This study has been designed to evaluate whether atorvastatin suppresses atherosclerotic lesion progression and inflammation in ApoE*3-Leiden mice beyond cholesterol-lowering *per se* under conditions which are relevant for the human situation.
ApoE*3-Leiden mice were fed a high cholesterol (HC) diet until mild atherosclerotic lesions had formed. HC diet feeding was either continued; or mice received a HC diet supplemented with 0.002%(w/w) atorvastatin (HC+A), a concentration which modestly lowered plasma cholesterol by 19%; or mice received a low cholesterol (LC) diet to establish a plasma cholesterol level similar to that achieved in the HC+A group.
Atorvastatin and hypocholesterolemic diet feeding both significantly reduced but did not block lesion progression to the same extent, as assessed by measuring total atherosclerotic lesion areas, lesion severities, and macrophage and smooth muscle cell areas. Furthermore, atorvastatin but not the hypocholesterolemic diet suppressed aortic VCAM-1 expression and, parallelly, monocyte adhesion. The expression of other endogenous inflammatory markers (MCP-1, MIF, PAI-1, MMP-9) and a transgene-expressed one (human CRP) was not additionally affected by atorvastatin, likely because the drug does not exert direct anti-inflammatory activity at low doses.
In conclusion, atorvastatin at low, therapeutical concentrations retards atherosclerotic lesion progression predominately via its cholesterol-lowering activity.
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