cells and we purified the recombinant enzymes by using different chromatographic strategies. For both lipases, the enzymatic assays were designed with fluorogenic surrogate phospholipids and triglycerides. The reactions were assembled through a homogeneous two-step procedure in 384 MTP format with a final reaction volume of 30 µl and monitored following the kinetic increase of fluorescence intensity.

Results: Recombinant human HL and sPLA2-IIa were produced in a catalytically active form. The reaction conditions were optimized to maximize the enzymatic activities in the miniaturized format and the kinetic constants for the fluorogenic substrates were determined. Specific inhibition in the HTS assay conditions was proved by using reference inhibitors.

Conclusions: The successful approach adopted to set up the homogeneous fluorescence-based enzymatic assays for HL and sPLA2-IIa in 384 MTP format represents an important progress to convoy these two therapeutically relevant lipases into the drug discovery process. The designed conditions are directly adaptable to 1536 MTP format and fully compatibility with an automated robotic procedure. In addition, this strategy can be potentially applied to develop HTS-compatible assays for other members of this important class of enzymes.

W10-P-007 TRANSFER OF LIPIDS TO HDL IN PATIENTS WITH CORONARY ARTERY DISEASE

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Objective: Lipoproteins are constantly being remodeled and transfer proteins such as CETP and PLTP shift cholesteryl esters (CE), phospholipids (PL) and triglycerides (TG) from one to other lipoprotein class. Free cholesterol (FC) can freely diffuse from the lipoprotein particles although PLTP may accelerate its transfer. The relationships between transfer of lipids and atherogenesis are complex and yet unclear. In this study, we developed a simple method of measuring lipid transfer to HDL *in vitro* and verified the transfer rates from an artificial lipoprotein model to HDL in subjects with or without coronary artery disease (CAD).

Methods: An artificial lipidic microemulsion labeled with ^{3}H-TG and ^{14}C-FC or ^{3}H-CE and ^{14}C-PL was incubated with 200µl plasma. After precipitation of apoB-containing lipoproteins and the microemulsion, the supernatant containing HDL was counted for radioactivity. 20 non-diabetic CAD patients aged 63±10 years and 22 paired controls without CAD were studied.

Results: Lipids transfer from the microemulsion to HDL (in % of total radioactivity/10 mg HDL-c)

	TG	FC	CE	PL
CAD	2.2±0.2*	2.0±0.2*	3.0±0.4*	6.0±0.5
Control	0.7±0.04	1.1±0.08	1.7±0.07	4.9±0.2

*$p<0.0001$, compared to Control.

Conclusions: Transfer of all lipids, except PL, to HDL is increased in CAD. Due to HDL important antiatherogenic roles, this result can be relevant to establish new mechanisms and risk factors in CAD.

W10-P-008 EFFECT OF ROSUVASTATIN AND ATORVASTATIN TREATMENT ON LPAI AND LPAI:AII IN PATIENTS WITH CORONARY ARTERY DISEASE AND LOW HDL CHOLESTEROL

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Objective: Low concentrations of plasma HDL cholesterol (HDLc) are an independent risk factor for coronary artery disease (CAD). It has been repeatedly shown that atorvastatin only results in a small increase in plasma HDLc. The question remains whether rosuvastatin results in a higher increase in plasma HDLc levels as compared to an equipotent dose of atorvastatin.

Methods and materials: RADAR is a randomized, multicenter, parallel-group study focussed on the effects of rosuvastatin (RSV) versus atorvastatin (ATV) on plasma HDLc and HDL apolipoproteins in 80 patients diagnosed with low HDL cholesterol levels (<1 mmol/L) and established CAD. Patients were randomized to receive 10 mg RSV or 20 mg ATV for 6 weeks, followed by up-titration to 20 mg RSV/40 mg ATV for 6 weeks and finally up to 40 mg RSV and 80 mg ATV for 6 weeks. HDLc and apo AI were analysed using standard procedures. LpAI and LpAI:AII were analysed using immunoelectrophoresis.

Results: Baseline plasma HDLc was 0.77 mmol/L (SD 0.09) (RSV) and 0.73 mmol/L (0.11) (ATV), apo AI: 0.99 (0.17) vs 0.91 L (0.14) g/L, Lp AI: 0.33 (0.09) vs 0.30 (0.06) g/L, and LpAI:AII: 0.66 (0.16) vs 0.61 (0.16) g/L. After 18 weeks of statin treatment HDLc was increased ($P<0.05$) in both the ATV (0.03, SD 0.11) and RSV group (0.02, SD 0.12). The difference between the 2 treatment arms, observed at the highest dose of statin medication, was not significant (p=0.61). Plasma apo AI levels were increased significantly after ATV (mean 0.10, SD 0.17) and RSV treatment (mean 0.11, SD 0.22)(both $P<0.001$). However, a trend towards a more beneficial improvement with RSV versus ATV was observed (P=0.06). Surprisingly, Lp AI levels decreased after both RSV (mean 0.02, SD 0.06) and ATV (mean 0.06, SD=0.06) (p=0.007). LpAI:AII levels increased by both statins to a similar extent (0.16 (SD 0.16) vs 0.13 (SD 0.21), $P<0.001$; RSV vs ATV: p=0.46).

Conclusions: RSV and ATV increase HDLc to a similar extent. In addition both RSV and ATV treatment lead to profound changes in HDL subfraction distribution.

W10-P-009 PREVALENCE OF CHOLESTERYL ESTER TRANSFER PROTEIN POLYMORPHISMS IN A BRAZILIAN POPULATION AND THEIR RELATIONSHIPS TO ATHEROSCLEROSIS

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Objective: To investigate the effects of cholesteryl ester transfer protein (CETP) TaqIB and I405V gene polymorphisms on early atherosclerosis, a low-density lipoprotein oxidation biomarker, the activity of regulatory proteins, lipid and lipoproteins parameters and carotid intima-media thickness (IMT).

Methods and Results: Two hundred and ninety-four volunteers, were enrolled in this study. The more prevalent genotypes were Taq B1B2 (45%) and 405 IV (49%). The waist circumference and age were higher in patients with 405 IV genotypes. Taq B1B2 showed higher Lp (a) levels than others. Genotype 405 II showed lower concentrations of HDL-C and 405 IV higher ApoAI than others. CETP activity was higher in Taq B1B1 and lower in Taq B2B2 carriers and higher in 405 II. PLTP activity was higher in 405 II genotypes. Autoantibodies to epitopes of oxidized LDL were reduced in 405 VV and in Taq B2B2 genotypes and higher carotid IMT was found in B2B2 genotype. The frequency of established cardiovascular disease was similar among the genotypes.

Conclusions: Different CETP polymorphisms elicited diverse responses of plasma lipids, lipoproteins, autoantibodies to oxidized LDL epitopes and carotid IMT.

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W10-P-010 DIFFERENT METABOLIC BEHAVIORS IN THE FASTING AND THE POST-ALIMENTARY PERIODS CLARIFY THE ASSOCIATION OF LIPEMIA WITH ATHEROSCLEROSIS MARKERS

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Objective: This work identified different metabolic behaviors to a standardized fat meal in early and late diet-induced triacylglycerol (TAG) responses, relating the latter to markers of atherosclerosis.

Design: Blood samples were collected before and after a liquid meal at 0, 2, 4, 6 and 8 hours (h) for different biochemical measurements; anthropometric data were collected.

Results: Sixty asymptomatic and normolipidemic adults were classified into early TAG responders to the test meal (n=39), late (n=21) and biphasic (among late responders, n=10). Reductions in HDL-cholesterol and insulin concentrations were observed in late and in biphasic responders in the TAG peak period and also in fast for HDL-chol as well as increases in free fatty acids in late responders in the TAG peak period. Post-alimentary CETP increase was absent in the biphasic group. Only late responders presented positive correlations between the carotid IMT and the TAG areas under the curves (AUC), TAG 0-8h, anti-oxi LDL, cholesterol, LDL-cholesterol and body mass index; also anti-oxi LDL correlated positively with cholesterol, and negatively with CETP, hepatic lipase and systolic blood pressure.

Conclusions: The association of atherosclerosis biomarkers with late post-alimentary lipemia could be due to a state of post-alimentary insulin resistance and of impaired reverse cholesterol.

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W10-P-011 HDL CHOLESTEROL-RAISING EFFECT OF RIMONABANT IN OVERWEIGHT/OBESE PATIENTS IS INDEPENDENT FROM BASELINE TRIGLYCERIDE LEVELS: EVIDENCE FROM RIO-LIPIDS

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Three large phase III clinical trials (RIO-Lipids, RIO-Europe and RIO-North America) have shown that rimonabant, the first selective endocannabinoid type1 (CB_1) blocker, represents a unique multitarget drug inducing not only significant weight loss/waist circumference reduction but also producing substantial improvements in metabolic risk factors such as plasma triglyceride (TG) levels, HDL-cholesterol and insulin sensitivity.

Objective: As the hypertriglyceridemic state of abdominal obesity is frequently accompanied by low HDL-cholesterol levels, the objective of the present study was to verify, in the RIO-Lipids trial, whether changes in HDL-cholesterol levels produced by rimonabant were independent of baseline TG levels.

Methods: The sample of 1036 patients of RIO-Lipids were stratified into three groups of fasting TG levels (<150, 150-199, ≥200 mg/dL) and their HDL-cholesterol response to 1-year treatment with rimonabant (20 mg/day) compared to placebo.

Results: Although patients in the lowest TG group had higher HDL-cholesterol levels than patients in the highest TG group, rimonabant at 20 mg/day nevertheless increased HDL-cholesterol by 22.5% in the lowest TG group (mean difference vs. placebo:+9.6%, p<0.001). A significant increase in HDL-cholesterol was also observed among patients in the second and third TG groups (mean difference vs. placebo:+8.8%, p=0.005 and +5.7%, p=0.011, respectively).

Conclusions: Thus, HDL-cholesterol was substantially increased by rimonabant 20 mg even in the absence of hypertriglyceridemia at baseline. Results from RIO-Lipids indicate that rimonabant increases HDL-cholesterol levels irrespective of baseline TG levels. These results are consistent with a direct effect of rimonabant on metabolic processes regulating plasma HDL-cholesterol levels.

W10-P-012 RELATION OF FER-HDL AND AIP TO CLINICAL AND LABORATORY PARAMETERS IN HATS

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Objective: Fractional cholesterol esterification rate in plasma depleted of apoB-containing lipoproteins (FER_{HDL}), a functional test of lipoprotein structure and size, correlated with coronary artery disease (CAD) participating in HDL-Atherosclerosis Treatment Study (HATS). We have now assessed the relation between the changes in coronary lesions with changes in plasma FER_{HDL} and log [TG:HDL-C], an atherogenic index of plasma (AIP), and lipoprotein particle size.

Methods: The HATS cohort included 160 patients with CAD and low HDL-C (< 0.9 mmol/L) who were treated over three years with one of four regimens: simvastatin plus niacin, antioxidants, simvastatin and niacin plus antioxidants (vits E, C, β-carotene, selenium), or placebo. FER_{HDL} was measured using a radioassay.

Results: The changes in coronary lesions correlated (Spearman) best with FER_{HDL} (r=0.308, p<0.0001 level. The mean values of FER_{HDL} and AIP changed significantly after 1 year of treatment in simvastatin-niacin and simvastatin-niacin-antioxidants groups (– 37% and –71%, and –31% and –55%, respectively). Spearman correlations between FER_{HDL} and AIP and apoproteins have been most significant in the order of non-HDL, apoC-lipoproteins (0.744 and 0.841), apoE (0.650 and 0.664), apoC lipoproteins in HDL (0.376 and 0.505), apoB (0.228 and 0.205), and apoAI (-0.203 and -0.240).

Conclusions: FER_{HDL} and AIP were among the best predictors of changes in coronary arteries in HATS. Both parameters were highly positively correlated with apoE, apoB, apoC apolipoproteins and inversely with apoA1. FER_{HDL} and AIP also reflected the size and concentration of lipoprotein particles.

W10-P-013 NOT ONLY ACTIVATION OF PPAR-ALPHA, BUT ALSO OF ANOTHER - SO FAR UNKNOWN FACTOR - IS NEEDED TO INCREASE APOA-I SYNTHESIS BY FENOFIBRIC ACID

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Objective: In human, fenofibrate increases apoA-I synthesis and consequently HDL plasma concentrations, which results in a decreased cardiovascular risk. Fenofibrate induced human apoA-I elevation does not occur in PPARα (-/-) mice, which indicates the essential role of transcriptional factor PPARα in apoA-I production. There are, however, other interventions that enhance apoA-I production often by unknown mechanisms. This underlines the need to further increase our understanding of regulatory pathways to elevate apoA-I production.

Methods: Human hepatic (HepG2 cells) and intestinal (INT407 or Caco-2 cells) cells were used to evaluate effects of fenofibric acid or synthetic PPARα agonists (GW7647 and WY14643) on apoA-I mRNA synthesis and protein secretion.

Results: In all three cell-lines, fenofibric acid increased apoA-I protein concentrations in a dose (0.1-0.3-0.6mM) and time-dependent (3-6-12h) manner. Simultaneous addition of fenofibric (0.6mM) acid and actinomycin D (transcription inhibitor; 5μg/ml), cycloheximide (translation inhibitor; 10μg/ml), or brefildin A (secretion inhibitor; 20μM), indicated that the fenofibric acid induced apoA-I synthesis seems to be regulated at transcriptional and translational level. Surprisingly, the synthetic specific PPARα agonists WY14643 (25μM) and GW7647 (100nM) increased PPARα mRNA concentrations, but not apoA-I mRNA synthesis or apoA-I secretion. In these experiments, RXR activation was not limiting as evaluated by simultaneous addition of 9-cis retinoid acid (10μM) with WY14643 or GW7647.

Conclusions: PPARα activation alone cannot explain fenofibric acid induced apoA-I secretion, suggesting a role for fenofibric acid induced transcriptional factors or co-activators or translational pathways.

W10-P-014 KNOCKDOWN OF HEPATIC ABCA1 DECREASES PLASMA HDL CHOLESTEROL LEVELS AND INFLUENCES POSTPRANDIAL LIPEMIA IN MICE

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Objective: ATP binding cassette transporter-1 (ABCA1) initiates the formation of mature HDL by facilitating apolipoprotein AI (apoAI) lipidation. In this study we investigated the impact of hepatic ABCA1 on systemic lipoprotein metabolism *in vivo* by an adenovirus RNA interference approach.

Methods: Efficiency of plasmid-based small interference RNA (siRNA)-mediated knockdown of co-transfected murine ABCA1 in HEK-293 cells was judged by RT-PCR, immunofluorescence and western blot analysis. The most effective plasmid was used to generate a recombinant adenovirus as a tool to selectively down-regulate ABCA1 expression in mouse liver.