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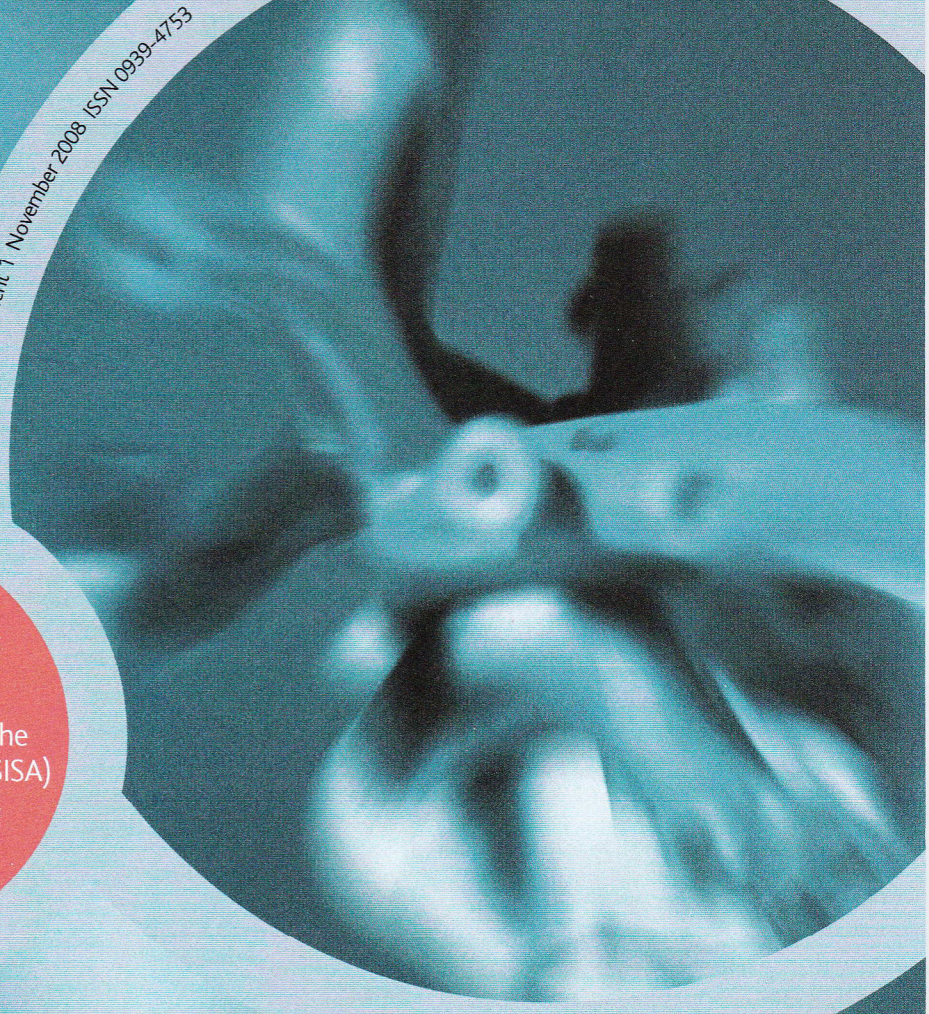

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never-smokers (6.26±3.21%) ($p < 0.0004$ and $p = 0.002$, respectively) but no differences were observed when the FMDvs.at-rest of light- and regular-cigarette consumers were compared ($p = 0.173$). Almost identical results were obtained when FMDvs.last-60'' were considered.
Conclusion: Light and regular cigarettes have the same detrimental effect of FMD, thus light cigarette consumption cannot be considered as a good alternative to smoking cessation.
Funding: Researches described were supported in part by Philip Morris USA Inc. and Philip Morris International.

5 CYTOKINE/CHEMOKINE DIFFERENTIATION PATTERN IN PATIENTS WITH ST-ELEVATION MYOCARDIAL INFARCTION (STEMI) ASSOCIATED WITH HIGH LEVELS OF CIRCULATING IL-6: EIGHTEEN CYTOKINES/CHEMOKINES ANALYZED SIMULTANEOUSLY WITH THE FLEX-SET CAPTURE BEAD ASSAY (CBA)

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Background: Preliminary analysis of the population enrolled in the international, case-controlled First Acute Myocardial Infarction (FAMI) study showed a 3-fold increase in circulating IL-6 in STEMI patients in comparison to controls.
Objectives: We assessed the possible clustering of patients' behavior by means of multiple biomarkers' measurements, in patients with very high levels or low levels of IL-6.
Materials and Methods: Eighteen cytokine/chemokines (IL-2, IL-4, IL-7, IL-1β, IL-10, VEGF, FAS-L, GM-CSF, TNFα, IL-12p70, INFγ, IL-8, IP-10, MIP-1α, MIP-1β, eotaxin, MCP-1 and MIG) were analysed simultaneously with the Flex-Set CBA. Assays were performed on a total of 308 serum samples obtained within 6 hours from the onset of chest pain as follows: 109 patients belonging to the fourth interquatile (IQ) of IL-6 levels ($IL6^{high}$ STEMI with IL-6 median 17.1 pg/ml, iq 11.9–29.8 pg/ml), 96 patients belonging to the first IQ of IL-6 levels ($IL6^{low}$ STEMI with IL-6 median 3.7; 2.7–4.6 pg/ml) and 103 controls (IL-6 median 3.1; 2.3–5.2 pg/ml).
Results: The $IL6^{high}$ STEMI versus $IL6^{low}$ STEMI group showed increased levels of 6 out of 18 analyzed cytokines: IL-10 (5.3 vs 2.4 pg/ml; $p < 0.001$), IL-8 (10.5 vs 7.2 pg/ml; $p < 0.001$), MIP-1α (4.4 vs 3.5 pg/ml; $p < 0.001$), MIP-1β (86 vs 63 pg/ml; $p < 0.05$), MIG (160 vs 81 pg/ml; $p < 0.001$), MCP-1 (100 vs 68 pg/ml; $p < 0.001$). Similar levels of all cytokines were found in $IL6^{low}$ STEMI and controls with the exception of IL-10 (2.4 vs 2.0 pg/ml; $p < 0.05$) which was increased in $IL6^{low}$ STEMI, and MCP-1 (68 vs 92 pg/ml; $p < 0.05$) which was decreased in $IL6^{low}$ STEMI. Independence analysis of cytokine distribution, taking into account the 6 cytokines and chemokines which were significantly different and the levels of IL-6 and C-reactive protein, showed a distinct cytokine patterns in the $IL6^{low}$ and $IL6^{high}$ STEMI groups ($P < 0.05$).
Conclusions: The multi-cytokine approach allowed us to detect an inflammatory pattern in $IL6^{high}$ STEMI patients, consistent with the hypothesis that some patients with STEMI have a multifaceted inflammatory component, identified by high levels of circulating IL-6. This subgroup of patients might benefit by immune-modulating therapy.

6 CYTOKINE AND RECEPTOR PATTERN OF ACTIVATED VERSUS REGULATORY T CELLS IN CORONARY ARTERY DISEASES

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Background: Regulatory T lymphocytes (Treg) play a key role in maintaining self tolerance and in suppression of pathological immune response. In atherosclerosis an unbalance between activated and regulatory T-cell activity might play a role in the developement, evolution and instability of plaques. In murine models a reduction in Treg results in increased atherogenesis.
Aims of the study: The aim of the study is to evaluate the balance between circulating activated and regulatory T cells in various manifestations of coronary artery diseases (CAD) and the receptor patterns of these cells. The cytokine profile in its activation (IL-6)/regulation (IL-10) balance as expressed by changes in IL-6 and IL-10 was also evaluated.
Materials and Methods: Treg percentage, identified by CD3+CD4dimCD25high panel within the CD3+CD4+ T-cell compartment, was assessed in the study by means of 4- and 5-colour flow cytometry with conjugate antibodies (CD3, CD4, CD25, CD69 and CCR5 the 5-colour panel; CD3, CD4, CCR5 and HLA-DR the 4-colour panel). Circulating levels of cytokines were assessed by the CBA-Flexset technology. We studied four groups of patients: 49 healthy controls with no evidence of coronary disease, 36 patients with Cronic Stable Angina (CSA), 34 patients with Unstable Angina/Non ST Elevation Myocardial Infarction (NSTEMI) and 29 patients with ST Elevation Myocardial Infarction (STEMI). The state of activation of CD4+ T cells was determined by measuring two different markers: CD69, a marker of early activation, and HLA-DR, a marker of late activation. The reliability of the Treg percentage was assessed by two different measurements at 1 and 3 months in patients and controls, respectively. All values are expressed as medians (25%-75% pct).
Results: The percentage of Treg was stable over time. Treg percentage was significantly increased in STEMI (6.2, 5.4–7.5) vs controls (5.4, 4.6–6.2), vs CSA (5.3, 4.5–6.4) and vs NSTEMI (4.7, 4.4–5.7); while in NSTEMI a not significant trend towards a overall reduction in Treg number was observed. The expression of CD69 was higher in STEMI (1.0, 0.7–1.8) vs NSTEMI (0.5, 0.7–1.4), $p < 0.05$. A significantly increased expression of HLA-DR in NSTEMI (9.2, 5.7–12.2) and STEMI (5.3, 2.4–6.5) was observed vs controls (2.4, 1.7–4.0) and vs CSA (3.2, 1.5–6.8), $p < 0.0001$. The balance between activation and regulation, expressed as the ratio of Treg and activation markers was altered in CAD groups showing an increased activation of T-cell response. Moreover a decreased CCR5 Treg expression was observed in STEMI (20.7, 13.5–32.8) vs controls (31.3, 23.3–39.2), $p < 0.05$; These data seem to indicate a decreased tissutal migration ability since CCR5 represents a chemokine receptor for Treg homing. We observed a 2-fold increase in IL-10 levels in STEMI (4.1 pg/ml, 2.4–12.7) vs controls (2.0 pg/ml, 1.6–2.2) and vs NSTEMI (2.0 pg/ml, 1.7–2.9), both $p < 0.001$. IL-6 showed an increased serum concentration both in NSTEMI (6.9, 4.5–16.5) and STEMI (6.7, 4.2–24.9) vs controls (3.1, 2.5–4.5), $p < 0.001$ for all. IL-10/IL-6 ratio, used to determine the cytokine suppression/activation balance, was decreased in NSTEMI (0.7, 0.3–1.0) vs controls (0.6, 0.4–1.2), vs CSA (0.5, 0.3–0.8) and vs STEMI (0.7, 0.3–1.0), $p < 0.001$ for all.
Conclusions: This study showed a pro-inflammatory unbalance both in T-cell response and in cytokine network in all CAD groups as compared to controls. These findings could help understanding a possible protective role of Treg in patients with various manifestations of coronary artery diseases. The variability we observed so far may reflect the complexity of the pro- and anti-inflammatory network which still needs to be explored further.

7 EXPANSION OF T-CELL RECEPTOR ζ^{dim} EFFECTOR T CELLS IN ACUTE CORONARY SYNDROMES

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Objective: The T-cell receptor zeta (TCRζ)-chain is a master sensor and regulator of lymphocyte responses. Loss of TCRζ-chain expression has been documented during infectious and inflammatory diseases and defines a population of effector T cells ($TCR\zeta^{dim}$T cells) that migrate to inflamed tissues. We assessed the expression and functional correlates of circulating $TCR\zeta^{dim}$T cells in coronary artery disease.
Methods and Results: We examined the expression of TCRζ-chain by flow cytometry in 140 subjects. Increased peripheral blood $CD4^+TCR\zeta^{dim}$T cells were found in patients with acute coronary syndromes (ACS, n = 66; median 5.3%, inter-quartile 2.6–9.1% of total $CD4^+$T cells; $p < 0.0001$) compared to chronic stable angina (CSA, n = 32; 1.6%; 1.0–4.1%) and controls (n = 42; 1.5%; 0.5–2.9%). Such increase was significantly greater in ACS patients with elevated levels of C-reactive protein (CRP ⩾2 mg/l: 7.7, 3.8–11.3%, n = 41) compared to patients with ACS and low CRP levels (<2 mg/l: 3.3; 1.7–7.7%, n = 25), $p = 0.003$, and it persisted after the acute event. Moreover, $TCR\zeta^{dim}$ cells were also more represented in ACS compared to CSA and controls within $CD8^+$T cell subset (4.0%, 2–5.7% vs 1.2%, 0.8–2.1% vs 0.6%, 0.2–1.9%; $p < 0.0001$), NK subset (5.1%, 2.4–7.5% vs 1.6%, 0.9–2.7% vs 0.5%, 1.3–3.8%; $p = 0.001$) and $CD4^+CD28null$ subset (67.2, 39.4–82.9% vs 29.2, 23.0–46.9% vs 20.9, 11.1–33.1%; $p < 0.0001$). Finally, $CD4^+$ and $CD8^+TCR\zeta^{dim}$T cells isolated from ACS displayed an enhanced transendothelial migratory capacity.
Conclusions: $TCR\zeta^{dim}$T cells, an effector T-cell subset with trans-endothelial migratory ability, are increased in ACS, and may be implicated in coronary instability.

8 THE METABOLIC SYNDROME DOES NOT ADD TO CAROTID ATHEROSCLEROSIS BEYOND THAT EXPECTED BY RISK FACTOR COUNTING OR RISK SCORING

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Objective: The aim of this study was to assess if the Metabolic Syndrome (MS) has any add-on effect on subclinical atherosclerosis beyond that expected by risk factors (RF) counting or risk scoring.
Methods: Intima-media thickness (IMT) of carotid arteries was assessed by using B-mode ultrasound in 1805 patients (56±13 y; 52% women) attending a cardiovascular prevention program. Patients with (cases) or without (controls) MS according to NCEP ATP III criteria were 1:1 matched for sex, age and either the number of conventional RF (Analysis 1) or the Framingham risk score (Analysis 2). For Analysis 1 not more than 2 components of the MS were accepted as RF in the control group.
Results: Case:control matches were 211 for Analysis 1 and 244 for Analysis 2. No significant differences in carotid IMTmean and carotid IMTmax were found between cases and controls in both analyses (Analysis 1: IMTmean 1.03±0.38 vs 1.07±0.37; IMTmax 1.90±0.96 vs 1.95±0.90, cases and controls, respectively; Analysis 2: IMTmean 1.03±0.36 vs 1.01±0.33; IMTmax 1.91±0.94 vs 1.83±0.81, cases and controls, respectively; all $p > 0.1$).
Conclusions: According to our results the metabolic syndrome does not add to the extent of carotid subclinical atherosclerosis beyond that expected by RF

counting or risk scoring. These findings do not support any particular armful synergism between components of metabolic syndrome in determining carotid atherosclerosis. Funding: No commercial funding is disclosed.

9 CARDIOVASCULAR RISK IN CHILDREN: APOB/APOA-I AND NON-HDL-CHOLESTEROL

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Objective: Atherosclerosis starts in childhood although clinical manifestations of cardiovascular disease (CVD) do not usually emerge before the middle age. Clinical studies have established that elevated cholesterol (TC) and triglyceride (Tg) levels, low high density lipoprotein-cholesterol (HDL-C) and raised Lipoprotein(a) [Lp(a)] levels are associated with increased CVD risk. Measurements of TC, LDL cholesterol (LDL-C) and HDL-C are widely recommended for CVD risk assessment. However many studies in adults have demonstrated that apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I) and their ratio have a greater prognostic value than LDL-C. Furthermore, it is increasingly being recognized that the non-HDL-C level is a simple and accurate index of CVD risk. Aim of the study was the evaluation of TC, LDL-C, apoB and apoA-I levels, apoB/apoA-I ratio and non-HDL-C in a cohort of children affected by primary dyslipidemia, divided in two groups according to the family history of CVD.
Methods: we studied 285 children aged 10.04±3.34 years (89 FH, 88 FCHL, 54 dominant Hypercholesterolemia, 40 Hyperlipoprotein(a), 11 Familial hypertriglyceridemia, 2 Hyperchilomicronemia, 1 Phytosterolemia) with a family history of dyslipidemia and/or premature cardiovascular disease and 74 controls (age 9.35±4.71 years). TC, HDL-C, Tg, apoB and apoA-I were evaluated after an overnight fasting. LDL-C was estimated using the Friedewald formula while non-HDL-C was calculated as TC minus HDL-C. Patients were divided in two groups according to a positive (group 1) or negative (group 2) family history of CVD.
Results: dyslipidemic children showed TC, LDL-C, apoB, apoB/apoA-I ratio and non-HDL-C levels significantly higher ($p < 0.001$) than controls. We found differences of non-HDL-C and apoB/apoA-I ratio levels comparing the two groups of patients, however they only approached the statistical significance resulting $p = 0.085$ and $p = 0.06$ respectively. Any difference of TC, LDL-C and apoB was detected.
Conclusion: The present findings indicate the prognostic value of childhood apoB/apoA-I ratio and non-HDL-C levels in evaluating CVD risk. Measurement of apolipoproteins (apoB and possibly apoA-I) should be routinely added to the standard lipid profile (TC, Tg, HDL-C) to assess the atherogenic potential of lipid disorders. This is particularly relevant to dyslipidemias characterized by an elevation in plasma triglycerides. The apoB/ApoA-I ratio and non-HDL-C represent an advantage over traditional lipid variables for risk prediction and especially apoB could also replace the standard 'lipid profile' as a target for therapy in at-risk patients.

10 IMPACT OF PERIPHERAL GHRELIN ADMINISTRATION ON BODY WEIGHT, ADIPOKINE PROFILE AND LIVER FAT IN HIGH-FAT DIET OBESE RATS

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Rationale: Ghrelin is a gastric orexigenic hormone whose plasma concentration declines in obesity, with an emerging role in the modulation of insulin action, mitochondrial-lipid metabolism and inflammation. Obesity is commonly characterized by hepatic triglyceride accumulation that can contribute to liver and body insulin resistance and is associated with cardiovascular disease. The potential metabolic impact of ghrelin administration in obesity models is unknown.
Methods: In a rodent model of high-fat diet induced obesity (DIO: Wistar male 3-month-old rats fed 25% fat diet for one month) we measured the effects of 4-day twice-daily subcutaneous ghrelin injection at a non-orexigenic dose (200 μg/injection: G) on: (a) food intake, total body weight and selected visceral and subcutaneous fat pads; (b) plasma hormonal-metabolic profile [insulin, glucose, free fatty acids (FFA), adipokines]; (c) hepatic mitochondrial oxidative capacity (citrate synthase enzyme activity), activated (phosphorylated) master regulator of lipid oxidative metabolism AMP-activated protein kinase (AMPK) and triglyceride content.
Results: Compared to control animals fed a standard diet, DIO gained ~10% excess body weight with heavier ($P < 0.05$) visceral (epidydimal and retroperitoneal) fat pads. Plasma free fatty acids and leptin/adiponectin ratio were also higher in DIO ($P < 0.05$) while no significant differences were observed in plasma glucose and insulin. Liver triglyceride content was higher in DIO in spite of comparable tissue mitochondrial enzyme activities and higher AMPK phosphorylation (all $P < 0.05$). In spite of superimposable cumulative food intake, G led to higher 4-day body weight gain compared to DIO (14±2 vs 7±3 grams: $P < 0.05$). Visceral fat pads were however comparable while subcutaneous interscapular fat was heavier in G, associated with lower plasma leptin/adiponectin ratio (all $P < 0.05$ vs DIO). Plasma FFA were also lower in G than DIO ($P < 0.05$ vs DIO and control), in the absence of changes in plasma glucose and insulin. Compared to DIO, G did not change liver mitochondrial enzyme activities and AMPK phosphorylation but it resulted in lower tissue triglyceride content, (all $P < 0.05$ vs DIO).
Conclusions: 4-day ghrelin administration at a non-orexigenic dose is associated with favorable metabolic changes in diet-induced obese rats. In particular, in spite of higher weight gain ghrelin is associated with potential preferential subcutaneous fat accumulation, lower leptin/adiponectin ratio and lower plasma FFA. These changes might contribute to reduce hepatic triglyceride accumulation, in the absence of changes in mitochondrial enzyme activities and AMPK activation. The data provide a rationale for further investigation of ghrelin as a potential treatment for DIO-associated fatty liver.

11 EZETIMIBE/SIMVASTATIN COMPARED WITH DOUBLING THE DOSE OF SIMVASTATIN IN HIGH CV RISK DIABETICS NOT AT LDL-C TARGET WITH SIMVASTATIN ALONE: A DOUBLE-BLIND, RANDOMIZED ITALIAN STUDY

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Objectives: to compare the efficacy and safety of ezetimibe (EZE) co-administered with ongoing simvastatin (SIMVA) vs doubling the dose of SIMVA in reducing the low-density lipoprotein cholesterol (LDL-C) concentration after 6 weeks of treatment in patients with type-2 diabetes mellitus (T2DM), primary hypercholesterolemia and coronary heart disease (CHD). Design: multicenter, randomized, double-blind, double dummy study.
Participants: Twenty-three Italian centers participated in the study. Ninty-three adult subjects with T2DM and CHD, on a stable daily dose of SIMVA 20 mg for at least 6 weeks prior to randomization, with LDL-C concentration ⩾2.6 mmol/L (100 mg/dL) and ⩽ 4.1 mmol/L (160 mg/dL) and triglycerides (TG) concentration <3.99 mmol/L (350 mg/dL) were included. Interventions: ezetimibe 10 mg + simvastatin 20 mg placebo (EZE/SIMVA 10/20 mg/day group) or ezetimibe 10 mg placebo + simvastatin 20 mg (SIMVA 40 mg/day group) for 6 weeks; both treatments were added on to existing treatment with simvastatin 20 mg.
Outcomes: the primary outcome was the mean percent change from baseline (randomization visit) to endpoint after 6 weeks in LDL-C concentration; secondary outcomes included: the percentage of subjects who achieved the LDL-C goal as defined by the NCEP ATPIII guidelines (<2.6 mmol/L; <100 mg/dL), the mean percent change from baseline in total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations, the evaluation of safety and tolerability.
Results: Eighty-seven subjects (37 in the EZE/SIMVA and 50 in the SIMVA group) were included in the ITT analysis. EZE/SIMVA 10/20 produced a significantly greater mean percent change from treated baseline compared with SIMVA 40 in LDL-C (–32.2% vs –20.8%; $p < 0.01$) and in TC (–20.6% vs –13.2%; $p < 0.01$). A greater proportion of patients (close to statistical significance) achieved an LDL-C goal <2.6 mmol/L with EZE/SIMVA 10/20 mg than with SIMVA 40 mg (78.4 vs 60%, OR = 2.81; $p = 0.05$). There was no statistically significant difference between treatment groups in the percent change of HDL-C (0.85% vs 0.80% in the EZE/SIMVA and in the SIMVA group respectively) and TG (–8.5% vs –1.8% in the EZE/SIMVA and in the SIMVA group respectively). The treatment with EZE/SIMVA 10/20 mg was generally well tolerated with an overall safety profile similar to that of SIMVA 40 mg.
Conclusions: in high-risk subjects with T2DM and CHD not at the recommended ATPIII LDL-C target with simvastatin 20 mg, switching to the combination of simvastatin with ezetimibe (inhibiting both the synthesis and the intestinal absorption of cholesterol) produces a greater reduction of LDL-C and gets a greater proportion of subjects to an LDL-C concentration <2.6 mmol/L after 6 weeks of treatment.

12 VASCULAR AND METABOLIC ACUTE EFFECTS OF ROSUVASTATIN COMPARED TO SIMVASTATIN IN UNTREATED DYSLIPIDEMIC DIABETIC PATIENTS

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The effects of statins on glucose metabolism are controversial. In particular, it is not clear if statins may affect insulin sensitivity besides LDL-cholesterol and vascular reactivity improvement in diabetic patients. The present randomized, double-blind trial has been direct to evaluate the acute effects of rosuvastatin compared to simvastatin on glucose control, insulin-sensitivity and endothelial function in diabetic patients with untreated dyslipidemia. Twenty non obese male subjects (aged 56±8, mean±SD) with type 2 diabetes in OAD treatment and dyslipidemia were given rosuvastatin 20 mg (Group R, n = 10) or simvastatin 20 mg (Group S, n = 10) daily for one month. The following data were collected at baseline and after follow-up (differences in mean values assessed by t-test for paired data): BMI, waist circumference, fasting glucose, HbA1c, lipid profile, hs CRP, fibrinogen, leptin, adiponectin, insulin sensitivity assessed by euglycemic hyperinsulinemic clamp and endothelial function evaluated by brachial artery reactivity technique (BART). At baseline, subjects in the two arms had comparable anthropometric parameters, were in good glycemic control (Group R: fasting glucose 139±24 mg/dl, HbA1c 6.4±0.6%. Group S: fasting glucose