SHOULD WE CONSIDER FAMILIAL COMBINED HYPERLIPIDEMIA IN PEDIATRICS?

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Background. Familial combined hyperlipidemia (FCH) is a common lipid disorder characterized by increased plasma total cholesterol, triglyceride, and apolipoprotein B-100 (ApoB) levels. Diagnostic critical points include age onset presentation and phenotype variability, as the lack of a specific marker. Aim of this study was to ascertain whether FCH expression can be considered since childhood.

Methods. Children (n. 676, 10.9±3.9 yrs mean±SD) showing dyslipidemia were screened for primary disorders and submitted to dietary Step I regimen, under a nutritionist counseling for at least 3 months, then reevaluated. Lipoprotein profile was detected by standard method, ApoB and ApoA1 by Immunoturbidimetric method (Olympus analyzer), Apolipoprotein E (ApoE) genotype by PCR plus reverse dot-blot (ApoE Strip Assay ITA, Vienna Lab). A three generations genealogical tree including lipoprotein pattern, clinical symptoms related to cardiovascular disorders and therapy was constructed to provide an definite kindred history. Criteria to consider FCH included familial phenotype IIA, IIB, IV, and its intrafamilial variability. Cardiovascular disorder (CVD) was evaluated as a possible related but not critical condition.

Results. Children (n. 134 [73M/61F], age 10.7±3.5 yrs, BMI 20±3.8 mean±SD) from 108 kindreds resulted affected by TC 218±34.5, Tg 118.5±76.7, HDL-C 53±12.7, LDL-C 140.9±29.9, ApoB 101±19.0, ApoA1 135±21.5 mg/Dl mean±SD levels. Apolipoprotein ε number of patients and its percentage allele distribution was: ε 3 ε 4=46(42.1%), ε 4 ε 4=43(32%), ε 4 ε 4=43(2.9%), ε 3 ε 2=2(2%), ε 2 ε 4=11(1%). CAD and Premature CAD were detected in 82/108 (76%) and 54/108 (50%) respectively.

Conclusions. Children showing primary dyslipidemia should be considered for FCH diagnosis when lipid levels exceed the 95th percentile, age and sex related when the family history is sug-gested. Aim of this study was to establish whether FCH expression can be considered since childhood.

ABSTRACT

SIMVASTATIN ATTENUATES PULMONARY FIBROSIS IN A MURINE MODEL OF SYSTEMIC SCLEROSIS

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Atherosclerosis is a multifactorial disease, in which oxidation, NO and smooth muscle cell (SMC) proliferation play essential roles. To control different steps of the pathology, we synthesized NO-donor molecules (furans), that NO-dependently relax rat aorta strips and we joined them with antioxidants or bisphosphonates (farnesylpyrophosphate synthase inhibitors), for the generation of NO-donor/antioxidant or NO-donor/antiproliferative hybrids. All these molecules inhibited SMC proliferation (with different potencies), unlike their parent antioxidant compounds (phenols, vitamin E/C, carnosine, edaravone, melatonin). Since part of this effect is due to the furoxan moiety, to understand its molecular basis, we blocked the position 4 of the furoxan ring by a phenyl group and we found that the inhibition of proliferation and vasodilatation paralleled with the electron-attractor capacity of the group in 3 (R). Extending the study to 3-Ph-4-R furoxans (interchanged groups in 3 and 4) and to their related furazans (unable to release NO), 4-Ph-3-R furazans were the most potent inhibitors of SMC proliferation. Furazans were not effect, suggesting that the opening of the ring is essential for the inhibition of cell growth. Since our data support that this antiproliferative effect may not directly be NO-dependent, we are a) performing in vitro experiments coadminis-trating furazans and PTIO (a NO scavenger) on SMC proliferation, b) trying to identify, by different proteomic approaches, S-nitrosylated cellular protein(s) involved in proliferative processes affected by furazans, as shown by Williams et al. (2009) regarding the effect of furazans on thioaeorxin glutathione reductase, in Schis-tosomes. Based on these results and after the comprehension of the mechanism of action, we think that furazans could be suitable NO-donor moieties to be coupled with antioxidants or other drugs affecting different aspects of the atherosclerotic process. In case, the effectiveness of these hybrids will be then validated in animal models.

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Background/Purpose. Simvastatin is best known for its antili-pidemic action due to its inhibition of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase. Inhibition of biological pre-cursors in this pathway also enables pleiotropic immunomodula-tory and anti-inflammatory capabilities. The antifibrotic effect of simvastatin has been shown in human lung fibroblasts. This study aimed to measure the effect of simvastatin in development of fibri-sis in a murine model of systemic sclerosis.

Methods. Systemic sclerosis (SSc) was induced in BALB/c mice by daily subcutaneous injections of HOCl for 6 weeks reproducing the Cochlin oxidant stress model of SSc. Mice (n=24) were randomized in three arms: HOCl (n=10), HOCl plus simvastatin (n=9) and vehicle alone (n=5). Statin treatment was initiated 30 minutes after HOCl subcutaneous injection and continued daily for the 6 weeks. Skin and lung fibrosis were evaluated by histological methods. The severity of lung fibrosis was assessed using the Ashcroft score, while dermal thickness was measured in histological samples.

Results. Injections of HOCl induced cutaneous and lung fibrosis in BALB/c mice as demonstrated by routine histological analysis. Simvastatin treatment both reduced skin thickness (p<0.001) and attenuated the histopathological change of HOCl-induced pulmo-nary fibrosis (p<0.001).
Conclusion. Simvastatin reduces the development of pulmonary fibrosis potentially modulating adverse lung parenchymal remodeling as shown by the reduced deposition of collagen in alveolar septae in this murine model. Simvastatin also reduces skin thickness in the model.

The histological evidence from these experiments suggests that, given the low 1 per million death rate from statin prescriptions and the high morbidity and mortality of SSc pulmonary disease, consideration of human trials is warranted to determine the potential safety and efficacy of simvastatin for treatment of pulmonary fibrosis.

SIMVASTATIN TREATMENT DOWNREGULATES PULMONARY EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN A MURINE MODEL OF SYSTEMIC SCLEROSIS

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Background/Purpose. Systemic sclerosis (SSc) is characterized by autoimmunity, widespread microangiopathy, and fibrosis. Vascular endothelial growth factor (VEGF) is the primary inducer and key mediator of angiogenesis; serum levels of the angiogenic factor VEGF are significantly elevated in patients with SSc and correlate with the severity of pulmonary hypertension.

Methods. SSc was induced in BALB/c mice by daily subcutaneous injections of HOCI for 6 weeks reproducing the Cochin oxidant stress model of SSc. Mice (n=24) were randomized in three arms: HOCI (n=10), HOCI plus simvastatin (n=9) and vehicle alone (n=5). Simvastatin treatment was initiated 30 minutes after HOCI subcutaneous injection and continued daily for the 6 weeks. Lung concentrations of VEGF and ERK (extracellular signal-regulated kinase) were analyzed by western blot analyses.

Results. Pulmonary VEGF expression is reduced by simvastatin treatment compared to HOCI group (p<0.001). Levels of extracellular signal-regulated kinase (ERK), downstream mediator of VEGF, were also lower in the group of mice treated with simvastatin when compared to HOCI treated mice (p<0.001).

Conclusion. Simvastatin reduces the pulmonary expression of VEGF and ERK in the oxidant stress (Cochin) mouse model of SSc. Further studies are needed to measure the potential organ-specific effect of statins in vascular remodeling.

LOW HDL CHOLESTEROL IS AN INDEPENDENT PREDICTOR OF CHRONIC KIDNEY DISEASE PROGRESSION

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Introduction. Chronic kidney disease (CKD) patients often present with reduced plasma HDL-cholesterol levels. Whether this reduction is an epiphenomenon or is involved in renal function decline is unclear. Thus we investigate the relation between HDL-C levels/function and CKD progression in patients with different degrees of CKD.

Materials and Methods. 176 patients with CKD (50.85 ± 29.26 mL/min) were recruited and followed for up to 84 months. Lipid profile, metabolic status and kidney function were evaluated at predetermined times. Furthermore age matched controls were selected from a large prospective study of the general population (n=453). Scavenger Receptor class B member 1 (SR-B1) and ATP-Binding Cassette transporter A1 (ABCA-1) dependent cholesterol efflux, indexes of HDL functionality, were measured in 19 end-stage renal disease (ESRD) patients and in sex and age-matched controls.

Results. Diabetes and hypertension were associated with lower GFR; indeed HDL-C levels (<50 mg/dL if female or <40 mg/dL if male) were inversely correlated with kidney function (β=−0.056, p<0.001) and, at follow up, they predicted an earlier entry in dialysis or doubling of creatininaemia (p=0.001). At multivariate analysis low HDL-C levels were the only lipid parameter that affected the worsening of the disease independently of the presence of diabetes (RR = 0.951 [0.917-0.986] 95% C.I., p=0.007) and increased the predictive accuracy when added to other risk factors (C-statistic: 0.732 [0.644-0.860] vs 0.729 [0.6294-0.829] 95% C.I.). Of note, HDL plasma levels were not correlated to SR-B1 mediated serum cholesterol efflux potential in patients with renal dysfunction.

Conclusions. CKD patients with low levels of plasma HDL-cholesterol have a poor disease prognosis. HDL functionality is not impaired in patients with altered renal function as determined by the cholesterol efflux capacity. These data provide evidence supporting the hypothesis for a role of HDL in determining CKD progression.
PLATELET AND LEUKOCYTE ROS PRODUCTION AND LIPOPEROXIDATION ARE ASSOCIATED WITH HIGH PLATELET REACTIVITY IN ACUTE CORONARY SYNDROME PATIENTS ON DUAL ANTIPLATELET TREATMENT

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ABSTRACT

High platelet reactivity (HPR) on dual antiplatelet therapy is a risk factor for adverse vascular events in acute coronary syndrome (ACS) patients. Several studies showed that reactive oxygen species (ROS) may be involved in modulating platelet function. The aims of the present study were to investigate, in patients with ACS:

1) the relationship between the ROS production by and lipoperoxidation and platelet activation;

2) the association of cellular ROS production and presence of HPR to ADP and arachidonic acid (AA) stimulation in patients receiving both clopidogrel and ASA therapy.

In 132 ACS patients, ROS production and lipoperoxidation in platelets and leukocytes were investigated by FACS analysis using the fluorescent probe H2DCFDA and BODIPY 581/591C11. Dual HPR by AA and ADP was defined in the presence of platelet aggregation by 1 mM AA≥20% and by 10µM ADP≥70%. Significantly higher levels of platelet, lymphocyte and–to a lesser extent–monocyte- and granulocyte-derived ROS were detected in dual HPR patients (n=49) with respect to non-HPR patients (n=49) (Platelet: 2412 (1255-3963) vs 997 (566-1901); Lymphocyte: 9365 (3273-15063) vs 6984 (3530-11046); Monocyte: 17739 (9266-29057) vs 15470 (7651-26436); Granulocyte: 23108 (10105-34954) vs 20381 (9792-36436), p<0.01). Similarly, platelet and mononuclear cell lipoperoxidation values were significantly higher in dual HPR with respect to non-HPR patients. No significant difference was found in granulocyte lipoperoxidation between the two groups of patients. At logistic regression analysis after adjustment for several potential founders, age, platelet- and leukocyte-derived ROS remained significantly associated to dual HPR (age: OR 95% CI-1.07 (1.05-1.12), p<0.01; platelet-ROS: 2.53 (2.3-5.0) p<0.001; lymphocyte-ROS: 1.22 (1.11-1.33), p<0.001; monocyte - ROS: 1.14 (1.06-1.23), p=0.001; Granulocyte-ROS: 1.04 (1.0-1.1), p=0.036). Platelet, lymphocyte and monocyte lipoperoxidation were also significantly related to dual HPR.

Our results demonstrate that in patients with ACS on dual antiplatelet therapy ROS production by and lipoperoxidation of platelets are strictly correlated to the different pathways of platelet aggregation and that ROS production and lipoperoxidation of platelets and leukocytes are predictors of nonresponsiveness to dual antiplatelet treatment.

AGING AND PERIPHERAL ARTERY DISEASE INDEPENDENTLY PREDICT ULTRASOUND-DETECTED CAROTID STENOSIS IN CARDIAC SURGERY PATIENTS WITH NO HISTORY OF CEREBROVASCULAR DISEASE

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Objectives. Reduction of perioperative complications in cardiac surgery occurred thanks to EuroSCORE system, a validated method for calculating predicted operative mortality of patients un-
PENTraxin 3 and arterial thrombosis

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Long pentraxin 3, a member of the pentraxin family, is an acute phase protein associated with the outcome of cardiovascular diseases and atherosclerosis (1). Previous studies showed that PTX3 deficiency in Apo E KO mice is associated with the development of a larger atherosclerotic lesions and a pronounced inflammatory profile in the vascular wall (2). The aim of this study was to investigate the potential role of PTX3 in arterial thrombosis.

Following arterial thrombosis induction, generated by carotid topical application of FeCl\textsubscript{3}, PTX3 KO mice showed a significant reduction in carotid artery blood flow with a greater thrombus formation compared to WT mice. This effect was independent of altered hemostatic environment (tail bleeding time, fibrinogen plasma levels and platelet count), or of an impaired platelet activation since the expression of the activation’s markers P-selectin and integrin \( \alpha_\text{IIb}\beta_3 \) (after stimulation with collagen, the analogous of thromboxane U46619 and thrombin) and the survival curve in a model of lung thrombo-embolism was superimposable between PTX3 KO and WT mice.

PTX3 is released acutely following neutrophils degranulation, binds P-selectin, inhibits leukocytes rolling on endothelium (3) and reduces platelet-leukocyte interactions after MI (4). To investigate whether these mechanisms could be involved in the phenotype observed following FeCl\textsubscript{3} injury, a series of experiments were carried out in P-selectin KO/PTX3 KO mice. P-selectin KO/PTX3 KO mice showed a significant reduction in carotid artery blood flow and increased arterial thrombus formation compared to P-selectin KO, thus suggesting that PTX3 protection could go beyond P-selectin modulation.

Furthermore, an increase body of evidence points to PTX3 as an important component of the ECM where it can interact with some of its elements (5), consequently current studies are focused on the investigation of the relevance of PTX3 of vascular origin versus hematopoietic origin in the effect observed.

References
ABSTRACT

EFFECTS OF CIGARETTE SMOKE CONDENSATE ON THE TRANSMIGRATION OF HUMAN MONOCYTES

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Circulating monocytes are a key component of the atherogenic process: they adhere to the endothelium and migrate into the underlying intima in response to chemokines such as MCP-1 and RANTES. Once recruited into the atherosclerotic plaque, they differentiate to macrophages and contribute to its growth. Cigarette smoke (CS) is a risk factor for atherosclerosis, but it is not clear how CS affects monocyte behaviour in the atherosclerotic lesion. The aim of our study was to evaluate the effects of CS condensate (total particulate matter, TPM) on human monocytes (HM) transmigration through an endothelial cell layer. Endothelial cells EA.hy926 were grown on transwell filter inserts and activated with TNF alfa for 72 h while HM were isolated from peripheral blood of healthy non-smoker volunteers using the Ficoll Paque technique. The transmigration assay was performed under three different conditions. Pre-treatment with TPM for 24 h caused a slight decrease in HM transmigration rate (-18% vs control, p<0.05); this results are in agreement with our previous experiments conducted with the Boyden chamber on a collagen coated polycarbonate membrane. On the contrary, direct exposure of both HM and EA.hy926 cells to TPM for 6h in the transwell insert increased (+25% vs control, p<0.05) the transmigration of HM. An even more evident enhancement of monocytes transmigration was obtained with the exposure of both HM and EA.hy926 cells to medium conditioned by HM previously incubated with TPM (+256% vs control, p<0.001). These results suggest that TPM might affect different aspects of the atherogenic process: on one hand cells exposed to TPM could release chemotactic factors which amplify the recruitment of inflammatory cells into the plaque; on the other hand, long-term exposure to TPM could reduce monocyte mobility by impairing their egression from the plaque.

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ZOFENOPRIL AND RAMIPRIL PLUS ASA IN POST-MYOCARDIAL INFARCTION PATIENTS WITH LEFT VENTRICULAR SYSTOLIC DYSFUNCTION: A SMILE-4 STUDY RETROSPECTIVE ANALYSIS IN HYPERCHOLESTEROLEMIC PATIENTS

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Background. Combination of an ACE-inhibitor (ACEI) and acetyl salicylic acid (ASA) is a therapeutic option for the cardiac patient. However, the pharmacological interaction between ACEI and ASA may abate ACEI efficacy on cardiovascular outcomes. In the SMILE-4 Study zofenopril + ASA was more effective than ramipril + ASA on prevention of 1-year occurrence of major cardiovascular events in patients with acute myocardial infarction (AMI) complicated by left ventricular dysfunction (LVD).

Objective. To compare zofenopril and ramipril (+ ASA) efficacy in a subgroup of patients of the SMILE-4 Study with and without a history of hypercholesterolemia at enrolment.

Methods. The SMILE-4 was a phase IIIb, randomized, double-blind, parallel-group, multicenter, European study comparing the safety and efficacy of zofenopril 60 mg/day and ramipril 10 mg/day plus ASA 100 mg/day, in patients with LVD following AMI. The primary study end-point was 1-year combined occurrence of death or hospitalization for cardiovascular causes. Information on hypercholesterolemia at baseline was available in 515 out of the 716 patients of the intention-to-treat population.

Results. In the main study population the baseline rate of major cardiovascular events was significantly reduced by zofenopril vs. ramipril (odds ratio, OR and 95% confidence interval, CI: 0.70, 0.51-0.96; p=0.028). Overall, 140 (27%) patients had a positive history and 375 (73%) a negative history of hypercholesterolemia. The rate of major cardiovascular events was lower under zofenopril than under ramipril in the patients with hypercholesterolemia (35% vs. 39%; OR: 0.86, 0.43-1.70; p=0.670) and even more so in those without hypercholesterolemia (28% vs. 38% ramipril; OR: 0.61, 0.40-0.95; p=0.026). The reduction in the risk of major cardiovascular events was similar in patients with and without hypercholesterolemia.

Conclusions. This retrospective analysis of the SMILE-4 Study confirmed the good efficacy of zofenopril plus ASA in the prevention of long-term cardiovascular outcomes also in the subgroup of patients with hypercholesterolemia.

EFFECTS OF BIFIDOBACTERIUM SUPPLEMENTATION ON PLASMA LIPID PROFILE IN DYSLIPIDEMIC CHILDREN

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Introduction. Probiotics, a dietetic supplement as viable microorganisms, could act on lipid metabolism. Preclinical scientific evidences support the use of probiotics in the treatment of hypercholesterolemia, but clinical evidences are often contrasting. The aim of this study was to evaluate the effects of three strains of probiotics of Bifidobacterium on lipid profile in children affected by primary dyslipidemia.

Material and Methods. 37 dyslipidemic children, aged 10.8±2.1 yrs, were enrolled in a pilot prospective study. After a 4 week diet-run in period, children were randomized to receive a dietary supplement containing 3 strains of probiotics of Bifidobacterium or placebo for three months. After this period children who showed a decrease of lipid profile continued with probiotic supplementation for further three months. A dietary evaluation was performed by a dietitian who instructed patients and examined the weekly dietary diary they provided at any visit. Lipid profile (TC, HDL-C, TG) was assessed at baseline and after each treatment period by automatic analyzer (Olympus AU 2700, Japan).

Results. Baseline lipid profile examination showed: TC 223.4±23.3 mg/dl, HDL-C 50.4±11.8 mg/dl, TG 93.1 (37-184), HDL-C 148.4±20.9 mg/dl. After three months of treatment 16 children showed a decrease of lipid profile (p=0.89%). Probiotics reduced TC by 12.3% (p<0.05) and HDL-C by 10.7% (p<0.05) compared to placebo. This children group underwent the supplementation for additional three months. Data analysis concerning lipid profile are in progress. A strict dietary control demonstrated that no significant change occurred through the study. No adverse effects were signalled.
Conclusions. The treatment with three strains of Bifidobacterium was well-tolerated and it should be hypothesized that hypercholesterolemic children could benefit from this approach, when preliminary results will be confirmed also by additional larger controlled studies. The variability observed among responders should be related to the colonization time.

LIPOPROTEIN(a) LEVELS IN A POPULATION OF ITALIAN CHILDREN WITH FAMILIAL

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Aim. Increased serum Lipoprotein(a) (Lp(a)) levels are strongly associated with cardiovascular disease (CVD) in adults. Aim of the study was to assess the association between Lp(a) levels and family history of CVD in children with isoezogenous familial hypercholesterolemia (FH) at their first access to our Lipid Clinic.

Methods. 132 FH children (genetical diagnosis), 2-17 years of age (median 8), 60 males and 72 females, were evaluated for: family history of CVD (according to AAP guidelines 2008), anthropometrical measures, pubertal stage (Tanner =1 vs >1), blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), Triglycerides (TG) by enzymatic method, Lp(a) levels by nephelometry. None was receiving pharmacological treatment for lipids.

Results. Lipid profile (mg/dl, mean±sd) was: TC 270±54, LDL 196±55, HDL 55±12, TG 77±38. Family history was positive in 71 (CVD+) children (first degree relatives in 21, second in 50) and negative in 61 (CVD-). The lipid profile was comparable in CVD+ and CVD-, p>0.1. There was no statistically significant difference in Lp(a) mean levels in CVD+ vs CVD-group (20±23 vs 22±25, p>0.1) nor in pubertal stage <1 or=1 (22±25 vs 18±22, p>0.1). Blood lipid profile was not different by age, sex, pubertal stage.

Conclusions. The correlation between Lp(a) levels and family history for CVD in the group considered is weaker than reported. The correlation between Lp(a) levels and family history of CVD in children with heterozigous familial hypercholesterolemia (FH) at their first access to our Lipid Clinic.

Background. Cerebral Autosomal Dominant Arteriopathy with Subcortical Infaracts and Leukoencephalopathy (CADASIL), is an inherited disease due to cerebral microangiopathy presenting with variable pictures, including stroke, progressive cognitive impairment and disability. Among pathogenic processes, endothelial dysfunction has been hypothesized.

Objective. To evaluate the role of biomarkers of endothelial dysfunction (EPCs and CPCs, von Willebrand factor (vWF), and thrombomodulin (TM)) in a large CADASIL series.

Methods. 45 CADASIL patients and 33 sex and age-matched controls were enrolled. EPCs were measured by using flow cytometry and defined as positive for CD34 and CD133; CPCs as positive for CD34, CD133 and CD45. vWF and TM were measured using commercially available ELISA kits.
Results. In comparison with controls, Cadasil patients presented significantly lower EPCs levels (CD34/KDR:0.07 vs 0.1 cells/µl, p=0.09; CD133/KDR:0.07 vs 0.1 cells/µl, p=0.008; CD34/CD133/KDR:0.05 vs 0.1 cells/µl, p=0.002), significantly higher vWF activity (130.2 vs 91.7%, p=0.014) and similar levels of TM (29.2 vs 27.4 ng/ml, p=0.955).

CPCs were not significantly lower in Cadasil, but patients with stroke or dementia had significantly reduced CPCs levels than patients without (CD34:1.85 vs 2.83 cells/µl, p=0.014; CD133:1.77 vs 2.78 cells/µl, p=0.009; CD34/CD133:1.77 vs 2.73 cells/µl, p=0.009). Both vWF and TM were not significantly higher in the same subgroup of Cadasil patients with a more severe phenotype.

Conclusions. This is the largest series of Cadasil patients in which biomarkers of endothelial dysfunction have been studied. We confirmed the previously reported association between EPCs and CPCs and the disease, and we found an association with vWF, supporting the hypothesis of the presence of endothelial dysfunction in this disease and its potential role in modulating phenotype.

IMPACT OF PHYSICAL EXERCISE AND INFLAMMATORY STATE ON ENDOTHELIAL PROGENITOR CELLS IN ACUTE CORONARY SYNDROME PATIENTS ATTENDING A CARDIAC REHABILITATION PROGRAM

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Purpose. Among the benefits of a cardiac rehabilitation (CR) program for patients after an acute coronary syndrome (ACS) there is the mobilization of endothelial progenitor cells (EPCs). However, not all patients respond to CR with an increase of EPCs. We performed this study to identify the characteristics of patients who will not benefit from the increase of EPCs at the end of the CR program.

Methods. 112 ACS patients were admitted to a four-weeks CR program. EPCs, high sensitivity C-reactive protein (hsCRP) and NT-ProBNP levels were determined at the beginning (T1) and at the end (T2) of the CR program. All patients performed a cardio-pulmonary exercise test at T1 and at T2. EPCs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+. hsCRP and NT-ProBNP were measured by nephelometric and immunometric method, respectively.

Results. At T2 we observed a significant increase of EPCs (p=0.001), VO2 peak, Watt max HDL-cholesterol (p=0.0001) and a significant decrease (p=0.001) of hsCRP and NT-ProBNP; triglycerides, HbA1c, systolic blood pressure and waist circumference. Moreover, variations of VO2 peak were significantly correlated with the variations of EPCs. Patients with increased EPCs showed significantly (p=0.01) lower baseline levels of CRP and higher baseline TM levels. In a multivariate logistic regression analysis, the lowest tertile of baseline hsCRP significantly affected the likelihood of having an increase of EPCs at the end of the CR program.

Conclusions. A CR program determines an increase of EPCs with a decrease of CRP and NT-ProBNP. A different trend for EPCs can be detected among patients correlated to CRP levels and exercise tolerance.
THE PUTATIVE CANNABINOID RECEPTOR (GPR55) IS A NOVEL RECEPTOR REGULATED BY OXLDDL IN HUMAN MACROPHAGES

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Atherosclerosis is a progressive, chronic inflammatory disease which is the primary cause of morbidity and mortality in the western world and whose identification of promising novel therapeutics is thus of great interest. The endocannabinoid system (ECS) comprises a class of endogenous bioactive lipids, the cannabinoid receptors 1 and 2 (CB1R and CB2R) and the enzymatic machinery deputed for endocannabinoids metabolism. The role of cannabinoid receptors in atherosclerosis is still controversial: while CB1R is closely associated with cardiometabolic risk factors, including obesity and increased serum lipid production, the CB2R seem to bear a protective and anti-inflammatory role, downregulating both innate and adaptive immune responses. However, some of the well-documented non-CB1R, non-CB2R effects of certain cannabinoid ligands have recently attributed to the orphan G protein-coupled receptor GPR55, alias the “purported CB3” receptor. Given the vast therapeutic potential of the cannabinoid system, the revelation of a novel cannabinoid-sensitive target was treated with great excitement. Intriguingly, GPR55 function was first studied in the endothelial cells of vasculature and its role was associated with several physiological processes, including vascular relaxation, glucose homeostasis and immune responses. Our work investigates for the first time the expression and the effects exerted by GPR55 in human atherosclerotic cells, inasmuch as we observed an up-regulation of this receptor in oxLDL-enriched human macrophages compared to control macrophages, and its stimulation with the agonist O-1602 led to an increased CD36-mediated intracellular lipid droplets accumulation. Furthermore, the activation of GPR55 exerted a relevant immunomodulatory effect, inducing a proinflammatory cytokine profile characterized by heightened TNF-α, IL-12, and IL-6 production. Taken together, we provide evidence that GPR55 is indeed a novel biomarker of atherosclerosis and our data suggest that this putative novel cannabinoid receptor, originally implicated in neuropathic and inflammatory pain as well as bone remodeling, may be also involved in lipid accumulation and inflammation during atherosclerosis.

PLASMA AND ERYTHROCYTE FATTY ACIDS ASSOCIATION WITH LDL-CHOLESTEROLEMIA, BLOOD PRESSURE, AND BODY MASS INDEX IN A LARGE SAMPLE OF HEALTHY SUBJECTS: DATA FROM THE BRISIGHELLA HEART STUDY

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Background. Plasma and erythrocyte fatty acids represent the ability of the body to absorb and metabolize fatty acids, and they are only partly related to the dietary habits. The aim of our study was to evaluate the association between a large number of plasma and erythrocyte fatty acids and LDL-cholesterolemia, blood pressure and body mass index in a large sample of healthy subjects from the historical cohort of the Brisighella Heart Study, a well known epidemiological study on the main cardiovascular disease risk determinants (1972- still in progress). Methods: For this study we dosed a panel of plasma and erythrocyte fatty acids (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3 (w6), 18:3 (w3), 20:3, 20:4, 22:4, 22:5, 22:6) in 716 healthy men and 812 healthy women (mean age: 58.6+/-17.1).

Results. In an age and BMI adjusted model plasma 16:1 was significantly associated to MAP (OR 0.31; 95%CI: 0.27-0.35), plasma 22:5 and LDL-C (OR 7.60; 95%CI: 2.90-14.25; OR 6.11; 95%CI: 2.43-10.69), respectively, plasma 20:5 to LDL-C (OR 0.96; 95%CI: 0.60-1.32, respectively) while both erythrocyte and plasma 20:3 was associated to BMI (OR 0.38; 95%CI: 0.24-0.59), plasma 14:0 directly (OR 7.21; 95%CI: 2.90-11.52) and 18:3 (w3) inversely (OR -0.21; 95%CI: -1.30 - 0.90) to TG, both erythrocyte and plasma 20:3 was associated to BMI (OR 0.38; 95%CI: 0.16-0.61; OR 0.96; 95%CI: 0.69-1.32, respectively) while only plasma 18:2 to BMI (OR -0.07; 95%CI: -0.95 - 0.35).

Conclusion. In a large sample of healthy pharmacologically untreated subjects, different erythrocyte or plasma fatty acids patterns are linked to different cardiovascular risk factors, but the size of the relationship is clinically relevant only as it regards plasma 22:4 and 22:5 and LDL-cholesterol and 14:0 and 18:3 (w-3) and TG.

HEPATIC STEATOSIS INDEX AND LIPID ACCUMULATION PRODUCT AS MIDDLE-TERM PREDICTORS OF INCIDENT METABOLIC SYNDROME IN A LARGE POPULATION SAMPLE: DATA FROM THE BRISIGHELLA HEART STUDY

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Background and aim. Non-alcoholic fatty liver disease is a large-ly prevalent condition associated to an increased risk to develop type 2 diabetes and cardiovascular disease. The aim of our study was to evaluate if some NAFLD index are able to predict the four year metabolic syndrome (MS) incidence in a large population sample of pharmacologically untreated subjects.
Methods and Results. From the general database of the Brisighella Heart Study, we selected a subsample of 824 pharmacologically untreated subjects without metabolic syndrome, type 2 diabetes, history of alcohol abuse or known liver diseases at the 2004 population survey and re-visited at the 2008 survey. We calculated for all the subjects the Hepatic Steatosis Index (HSI) and the Lipid Accumulation Product (LAP). Then we evaluated the predicting role of HSI and lnLAP for MS by the application of a Cox-regression analysis adjusted by the MS components.

Results. Considering the whole population, the best predictors of MS were age (OR 1.13, 95%CI 1.12-1.14, p<0.001), HSI (OR 1.17, 95%CI 1.14-1.21, p<0.001), and lnLAP (OR 1.16, 95%CI 1.15-1.17, p<0.001). When repeating the prediction analysis by sex, in men the best MS predictors were age (OR 1.13, 95%CI 1.11-1.14), Heart Rate (OR 1.13, 95%CI 1.11-1.15), and lnLAP (OR 1.17, 95%CI 1.15-1.18) in women the best predictors were age (OR 1.13, 95%CI 1.12-1.15), HSI (OR 1.20, 95%CI 1.15-1.26), and lnLAP (OR 1.16, 95%CI 1.14-1.17).

Conclusion. Both LAP and HSI, as index of NAFLD, are significant predictors of incident MS in a large sample of general population.

BERBERINE INDUCED IMPROVEMENT IN LIVER STEATOSIS INDEX IN OVERWEIGHT DYSLIPIDEMIC PATIENTS TREATED WITH LIPID-LOWERING NUTRACEUTICALS

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Background. Liver steatosis is a common feature of overweight dyslipidemic patients. We aimed at verifying the effect of short term treatment with berberine-monascus on liver steatosis index in overweight dyslipidemic patients with or without berberine.

Methods. 39 subjects (19 M, 20 W; mean age 60±11 years) affected by mixed dyslipidemia (LDL-C>130 mg/dL and TG>200 mg/dL) were randomized to treatment with a standardized lipid-lowering nutraceutical with or without berberine. Both LAP and HSI, as index of NAFLD, were measured at T0 and T8.

Results. All patients tolerated the treatment and no increase in transaminases was observed. Both treatment groups experienced a significant improvement of LAP (p<0.001) and HSI (p<0.001) but only the berberine-monascus treated patients experienced a significant improvement of HSI as well as with LAP (p<0.001). As regards the LSI, it did not change in monascus treated patients (T0= 36.2±2.0 vs T8= 35.3±1.4 p=0.05), while it significantly improved in the berberine-monascus treated ones (T0= 36.5±1.8 vs T8= 35.3±1.3, t= 4.750, p<0.001).

Conclusion. A short term treatment with berberine-monascus was well-tolerated in mixed dyslipidemia patients with liver steatosis and associated to a significant improvement in the liver steatosis index.

SEUM URIC ACID AND ARTERIAL STIFFNESS RELATIONSHIP IN A SAMPLE OF ADULT-ELDERLY SUBJECTS: DATA FROM THE BRISIGHELLA HEART STUDY

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Aim. To verify the relationship between serum uric acid (SUA) and arterial stiffness and subclinical atherosclerosis.

Methods. From the Brisighella Heart Study historical cohort, we randomized a sub-sample of 619 subjects (248 males), aged 53.5±11.2. Preclinical atherosclerosis was detected by carotid intima-media thickness (IMT) measurement. Arterial stiffness was determined by measuring the carotid-femoral pulse wave velocity (PWV) by means of a validated tonometer.

Results. A linear regression analysis showed a significant relationship between SUA and IMT (r=0.20, p<0.0001) and PWV (r=0.12, p<0.001). The age-adjusted relationship between SUA and PWV and IMT values was analysed. IMT was significantly higher in 3rd and 4th quartiles in relation with 1st and 2nd quartiles (p<0.001) (global trend: P<0.0001). With regard to aortic PWV values, these were higher in 3rd quartiles (p<0.05) and 4th quartiles (p<0.001) in relationship with 2nd quartile, but not with the 1st one (global trend: P=0.04). A stepwise regression analysis shown that SUA was one of the main factors affecting IMT (p=0.01) together with age (p=0.001), smoking (p=0.001), BMI (p<0.05), LDL-cholesterol (p<0.01), mean arterial pressure (p<0.01), and low level of heart rate (p<0.01). This model accounted for 42% of the variance in IMT. On the contrary, SUA was not an independent factor affecting PWV. The main factors affecting PWV (R2=42%) were age, BMI, heart rate, mean arterial pressure and low level of cholesterol LDL.

Conclusions. This study has shown an association between SUA high level and arterial stiffness and a possible independent role of SUA on atherosclerotic disease.

CHOLESTEROL CONTROL AND INCIDENT ANTIHYPERTENSIVE TREATMENT IN HYPERCHOLESTEROLEMIC SUBJECTS: A PHARMAEPEPIEDEMOLOGICAL REPORT

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Background. Preliminary evidence suggests that hypercholesterolemia is associated to an increased risk to develop hypertension. We aimed at evaluating the association between LDL-C level and the incidence of new antihypertensive treatment in a large population sample.

Methods. A population-based cohort of 20,074 adult subjects from an Italian Local Health Unit of Umbria Region with at least one LDL-C measurement between July 1, 2006 and June 30, 2007 and free of antihypertensive treatment (AHT) at baseline was followed from the LDL-C date until death or December 31, 2009. The co-
EVALUATION OF HOMOCYSTEINE AND LIPOPROTEIN(A) PLASMA LEVELS CAN REVEAL ADVANCED SYSTEMIC ATHEROSCLEROTIC BURDEN IN ACUTE CORONARY SYNDROME PATIENTS.

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Background. Strong evidence supported the association between high levels of homocysteine (Hcy) and Lipoprotein(a) (Lp(a)) and an increased rate of ischaemic vascular events. We investigated whether the assay of Lp(a) and hcy levels can improve the individual definition of systemic atherosclerotic burden over traditional cardiovascular risk factors (CRFs) assessment in acute coronary syndrome (ACS).

Materials and Methods. Study population consisted of 162 patients (F=30.9%; age 67.8±12.2 years) with acute coronary syndrome (ACS). All patients underwent medical examination, fasting blood sampling, ankle-brachial-index (ABI) measurement. Lp(a), hcy, complete blood count (CBC) and lipid profile, triglycerides and low levels of high-density lipoprotein cholesterol.

Results. During the mean follow-up of 1.6 years, 10.7% (n=1,166) of patients with LDL-C <130 mg/dL, 12.3% (n=772) of patients with LDL-C between 130 and 159 mg/dL, 12.2% (n=319) of patients with LDL-C between 160 and 189 mg/dL, and 13.9% (n=102) of patients with LDL-C ≥190 mg/dL started HPT. Compared with the lower LDL-C levels group, the LDL-C ≥190 mg/dL group showed the higher overall incidence rate (11.17 vs 6.03 for LDL-C <130, 9.33 for LDL-C between 130 and 159, 9.60 for LDL-C level between 160 and 189 per 100 person-years, P<0.001).

Conclusion. A better control of serum cholesterol levels seems to be associated to a significantly lower incidence of new HPT in a large cohort of general population.

NICOTINIC ACID/LAROPIPRANT ADDITION TO CHRONIC LIPID APERHERESIS IN VERY HIGH-RISK PATIENTS WITH ACUTE CORONARY SYNDROMES INFLUENCES RISING OF LIPOPROTEIN(A) PLASMA VALUES BETWEEN TWO CONSECUTIVE APHERETIC PROCEDURES

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Background. We investigated whether the chronic addition of nicotinic acid/laropiprant (2000 mg/20 mg per day) to lipid apheresis in very high-risk patients was able to influence rising rate of Lipoprotein (a) plasma value (Lp(a)) between two consecutive apheretic procedures.

Materials and Methods. The study population consists of 8 patients (F=3, M=5; age 64 (55-68) y.o.) with acute coronary syndrome, symptomatic peripheral arterial disease and elevated plasma Lp(a) levels. All patients underwent blood sampling: before (T0) and immediately after (T1) apheretic procedure, after 2 (T2), 4 (T3) and 6 (T4) days from baseline (T0). We compare Lp(a) levels at each control between apheresis-without-niacin/laropiprant period (A) and apheresis-during-niacin/laropiprant therapy period (AN).

Results. Lp(a) values at each control, between A and AN, were: 1205 (847-1750) mg/L, 275 (20-300) mg/L, 570 (411-880) mg/L, 1100 (816-1600) mg/L and 1080 (770-1320) mg/L, 160 (20-300) mg/L, 520 (300-680) mg/L, 550 (346-720) mg/L, 790 (640-1010) mg/L, respectively. Significant differences in Lp(a) plasma values, between A and AN, were found at T3 (p=0.048) and T4 (p=0.017) controls.

Conclusions. Niacin/laropiprant addition to lipid apheresis slows rising rate of Lp(a) plasma values between two consecutive procedures.

ABSTRACT
PERIPHERAL ARTERIAL TONOMETRY FOR ASSESSING ENDOTHELIAL FUNCTION IN RELATION TO DIETARY HABITS

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Background. Peripheral-arterial-tonometry (PAT) provides, with good reproducibility, measures of NO-mediated endothelial response, which correlate with flow-mediated-dilation (FMD) findings obtained by brachial artery ultrasound. Few data about the ability of exploring endothelial function by PAT in relation to dietary habits are available. Aim of this study was to evaluate RHI in subjects referring to for primary prevention, in relation to classical risk factors, in particular to adherence to Mediterranean diet and red wine consumption.

Materials and Methods. The study population comprised 95 consecutive clinically stable subjects in primary prevention for cardiovascular diseases. All subjects underwent medical questionnaire, clinical examination, and peripheral arterial tonometry for endothelial function evaluation.

Results. A significant inverse correlation between RHI values and BMI (r=-0.271; p=0.029) and the number of cardiovascular risk factors was found (r=-0.32; p=0.003). A significant positive correlation between score of adherence to Mediterranean diet and RHI values was found in subjects with RHI values >1.50 in comparison to others (39 (27-50) vs 33 (28-45), respectively; p=0.064). RHI values were significantly higher in regular drinkers (1.59 (1.28-2.29); 2.02 (1.38-3.54) respectively). Higher adherence to Mediterranean diet was found to be significantly associated with flow-mediated-dilation (FMD) findings (r=0.305; p=0.003). A significant positive correlation was found (r=-0.32, p=0.003). A significant positive correlation between RHI values and PAT values for mono-vessel, bi-vessel, three-vessel CAD patients in comparison to mono-vessel subjects (5.0 (2.0-9.4) µmol/L) (p value for mono- versus 2-vessel, p=0.0001; 2- versus 3-vessel, p=0.001; p for trend, p<0.0001). Hcy values were significantly higher in 2-vessel CAD patients in comparison to mono-vessel subjects (5.0 (2.0-9.4) µmol/L) (p value for mono- versus 2-vessel, p=0.0001; 2- versus 3-vessel, p=NS; p for trend<0.0001). Lp(a) values were significantly associated with CAD extension (Lp(a) values for mono-vessel, bi-vessel, three-vessel CAD: 20 (20-1130) mg/L, 332.5 (20-1680) mg/L, 670 (20-1828) mg/L, respectively; mono- versus 2-vessel, p=0.005; 2- versus 3-vessel, p=0.06; p for trend <0.0001). The contemporary presence of Lp(a) >300 mg/L, hcy >15 µmol/L and PAT<1.50 values was found in 12 (35.3%) 3-vessel, 2 (4.7%) 2-vessel, 9 (25.7%) mono-vessel CAD patients.

Conclusions. We showed that endothelial dysfunction and markers of atherosclerotic burden were associated to multi-vessel coronary involvement.

ENDOTHELIAL DYSFUNCTION AND MARKERS OF SYSTEMIC ATHEROSCLEROSIS ARE ASSOCIATED WITH THE EXTENSION OF CORONARY ARTERY DISEASE IN ACUTE CORONARY SYNDROME PATIENTS

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Background. Several studies showed the main role of endothelial dysfunction in the early stage and progression of vascular disease; Lipoprotein(a) (Lp(a)) and homocysteine (Hcy) were markers of atherosclerotic burden, linked to an increased rate of ischaemic vascular events.

Purpose of our study was to evaluate whether the alterations of endothelial function, investigated by peripheral arterial tonometry (PAT), and hcy and Lp(a) levels, may be associated with the extension of coronary artery disease (CAD) in high risk patients.

Materials and Methods. Study population comprised 87 patients (F=25.3%; age=67.8±12.2 years) with previous acute coronary syndrome (ACS), who underwent medical examination (cardiovascular risk factors (CRFs) evaluation), fasting blood sample (Lp(a), hcy), coronarography, and PAT.

Results. We found 10 (11.5%) mono-vessel, 34 (39.1%) bi-vessel and 43 (49.4%) three-vessel CAD patients. PAT values were significantly associated with progressive CAD extension (PAT values for mono-vessel, bi-vessel, three-vessel CAD: 1.91 (1.55-3.99), 1.64 (1.26-2.70), 1.40 (1.20-2.12), respectively; mono- versus 2-vessel, p=0.006; 2- versus 3-vessel, p=0.001; p for trend, p<0.0001). Hcy values were significantly higher in 2-vessel (1.5 (3.0-23.2) µmol/L) and 3-vessel (1.4 (3.0-23.2) µmol/L) CAD patients in comparison to mono-vessel subjects (5.0 (2.0-9.4) µmol/L) (p value for mono- versus 2-vessel, p=0.0001; 2- versus 3-vessel, p=NS; p for trend<0.0001). Lp(a) values were significantly associated with CAD extension (Lp(a) values for mono-vessel, bi-vessel, three-vessel CAD: 20 (20-1130) mg/L, 332.5 (20-1680) mg/L, 670 (20-1828) mg/L, respectively; mono- versus 2-vessel, p=0.005; 2- versus 3-vessel, p=0.06; p for trend <0.0001). The contemporary presence of Lp(a) >300 mg/L, hcy >15 µmol/L and PAT<1.50 values was found in 12 (35.3%) 3-vessel, 2 (4.7%) 2-vessel and none mono-vessel CAD patients.

Conclusions. We showed that endothelial dysfunction and markers of atherosclerotic burden were associated to multi-vessel coronary involvement.

AORTIC STENOSIS IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA WITH IVS10+1 G>A LDL RECEPTOR MUTATION: A CASE REPORT

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Case report. A 56 years old male with a history of familial hypercholesterolemia (FH), non smoker, presented to our hospital for a clinical examination. He showed mild dyspnea on exertion. The blood pressure was 130/80 mmHg, and the pulse rate was 70 beats/min. Physical examination showed A2 systolic ejection murmur (3/6 Levine) was heard in the second intercostals space on the right sternal border, radiated to the neck. Blood examination showed Total Cholesterol 400 mg/dl, HDL Cholesterol 60 mg/dl, LDL Cholesterol 314 mg/dl, in therapy with ezetimibe/simvastatin 40 mg. The electrocardiogram indicated left ventricular hypertrophy. Transthoracic echocardiography showed moderate aortic stenosis (mean gradient 38 mmHg, valve area 1.2 cm2), diffuse hypertrophy of left ventricular wall and preserved left ventricular ejection fraction. The patient underwent a coronary angiography with implantation of a stent on anterior descending artery. A genetic analysis was carried out for the detection of a LDL-receptor (LDLR) mutation, and a mutation (IVS10+1 G>A) was identified at heterozygous status. The patient started therapy with clopidogrel, aspirin, beta
HDL AND IMMUNE CELL RESPONSE: ROLE OF HDL ON MONOCYTES IN HUMANS AND ANIMAL MODELS

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IN HumANS AND ANImAL moDelS

HDl AND ImmuNe Cell ReSPoNse: lead to heart failure and sudden death.

An early genotypic identification could be useful to identify high risk mutation in order to an aggressive and effective therapy.

Conclusion. An early genotypic identification could be useful to identify high risk mutation in order to an aggressive and effective therapy for cardiovascular prevention. Aortic stenosis when present should be monitored by echocardiography because it might lead to heart failure and sudden death.

VITAMINA D E FATTORE DI RISCHIO CARDIOVASCOLARE IN PAZIENTI CON ARTERIOPATIA PERIFERICA

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Obiettivo. Scope of the present work is the analysis of relations present between variables of Vitamin D 25 OH, factors of risk CV and diffusion of the atherosclerotic plaque in 150 subjects affected from arterial periphery simontic.

Materiali e Metodi. The indagine is been conducted on 150 patients (104 M; 46 F), affected from arteriopathy periphery simontica, of età media pari a 71.2±7.1 years. The patients are been compared for evaluation of parameters biohumoral risk of cardiovascular disease, of markers of inflammation and activity of trombotica. Results showed a multifactorial condition of atherosclerosis, of immuno-cellular response. The population is been successfully divided in 4 classes to secondo status of Vitamin D 25 OH. The variables are been compared for metod ANOVA, with new variables with method Chi-Quadrado. There are been successively evaluated the correlation (Pearson) present between status of Vitamin D 25 OH and factors of cardiovascular risk.

Risultati. The total of the population in examine the 43,5% presented a carenza grave of vitamin D, the 24% a carenza lieue, the 13,5% an insufficient e the 10 the status of insufficiency. The patients with carenza grave present livelli significativamente elevation of cholesterol ofcolesterolato total, APO B e APO/A1 ratio (P<0.05), livelli significativamente superiori di D-Dimero e F1+2 (P<0.05) e livelli significativamente maggiori di Hs PCR e VES (P<0.05). The objects patients ofvitamina D are inolte resulted to be most frequentemente affected from microalbuminurie (33% vs 6,6% P<0.047). The test of correlation have evidence si correlation between status of Vitamin D 25 OH e Colesterolo Totale (R-0,209 P<0,011), APO B (R-0,211 P<0,010), B/A ratio (R-0,162 P<0,049), Fibrinogeno (R-0,176 P<0,033) Log D-Dimero (R-0,095 P<0,044), F1+2 (R-0,230 P<0,0001), HsPCR (R-0,211 P<0,01), VES (R-0,216 P<0,008). Non si are rilevate differences significative in the therapy farmacologic and in the severity of arteriopathy periphery.

Conclusioni. The patients with arteriopathy periphery present molto frequentemente carenza di Vitamin D 25 OH tale carenza semble correlare in maniera significativa with the presence of dislipidemia aterogena (elevati livelli circolanti di Apo B e B/A ratio elevato) with esaltata attività trombotica (elevati livelli di D-Dimero, Fibrinogeno ed F1+2) ed elevata attività infiammatoria (HsPCR e VES elevate). These data confirm the possibile ruolo del deficit of Vitamin D 25 OH in the gene and in the progression of the aterogena esplicato through various mechanisms.
ABSTRACT

LA DISTANZA PERCENTUALE DAL TARGET TERAPEUTICO PER LDL-C: DISTRIBUZIONE IN UNA POPOLAZIONE E SUA INFLuenZA SUllA MORTALITÀ. LO STUDIO BRISIGHELLA

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Introduzione. Il raggiungimento del target terapeutico per ogni fattore di rischio (FR) cardiovascolare è essenziale per ridurre il rischio cardiovascolare globale (RCVG).

Oggettivi. Valutare, in soggetti dello Studio Brisighella, in ragione della distanza percentuale dal target terapeutico (DPTT) per LCL-C determinato sulla base del RCVG calcolato con l’algoritmo COURE: 1) distribuzione percentuale della popolazione, 2) sopravvenienza a 10 anni.

Materiali e Metodi. Il campione comprende, 294 uomini e 283 donne, di cui 70 uomini e 58 donne costanti nella partecipazione al follow-up quadriennale e 224 uomini e 225 donne saltuari, seguiti nel decennio 1988-1998 (nel quale si è svolto un intervento educativo-nutrizionale). I soggetti, confrontabili peretà, PAS, PAD e TG, sono stati suddivisi in funzione di categorie di DPTT per LCL-C. Gruppo A) 0-15% (area d’intervento con approccio dieta/integrazione alimentari); gruppo B) 16-40% (area d’intervento con statine di primo livello); gruppo C) >40% (area d’intervento con statine di secondo livello e/o associazioni). Sono stati confrontati soggetti costanti (SC) ai controlli rispetto agli incostanti (SI). La sopravvenienza è stata valutata su tutta la popolazione per sesso e per categorie di DPTT utilizzando curve Kaplan-Meier.

Risultati. I soggetti non a target sono pressoché il doppio nei SI (34.8% M, 32.1% F) rispetto ai SC (17.9% M, 11.7% F). Nei maschi il 49.7% dei SC erano nel gruppo A, il 38.3% nel gruppo B, il 12.1% nel gruppo C. Nei SI la distribuzione era: 36.6% M, 49.9% M, 13.6%. Nelle donne la distribuzione era: 70.7% M, 28.2% F, 1.1%. Nelle SI 46.1% M, 49.3% F. Le curve di Kaplan-Meier, nei maschi, dimostrano una riduzione progressiva della sopravvenienza passando dal gruppo A al gruppo C. La differenza non si evidenzia nelle donne probabilmente per lo scarso numero di eventi registrati.

Conclusioni. I dati confermano come un controllo costante dei FR cardiovascolari possa determinare una riduzione della DPTT anche di un singolo FR (es. LCL-C). Inoltre, almeno negli uomini, la DPTT può rappresentare un indicatore di sopravvivenza a 10 anni.

FAMILIAL HYPERCHOLESTEROLEMIA: AN UPDATE OF THE GENETIC CHARACTERIZATION

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Introduction. Familial Hypercholesterolemia (FH) is a common autosomal dominant disorder characterized by high levels of total and LDL cholesterol. In general population, FH is found with a heterozygous frequency of 1/500 whereas homozygotes or compound heterozygotes occur 1 in 106 individuals and show severe cardiovascular complications since childhood. Mutations in the LDL receptor (LDLR) gene are the main cause of FH. Although the number of LDLR mutations is very high (more than 1100) genetic clusters of mutations have been reported in different countries (1). We aim to enlarge the genetic spectrum of FH mutations in the LDLR gene in a Southern Italy population.

Materials and Methods. We enrolled 268 patients with clinically diagnosed FH, of which 197 were unrelated. The promoter and the 18 exons of the LDLR gene were amplified by PCR and directly sequenced. To confirm splicing mutations, we performed RT-PCR analysis on the mRNA. For detection of large rearrangements, copy number quantification of the 18 exons of the LDLR gene was performed by the SALSA MLPA kit (MRC-Holland).

Results. Screening of the LDLR gene revealed mutations in 136/197 patients (mutation rate 69.0%). Among the 48 different mutations found, 6 mutations (2 splicing alterations and 4 nonsense mutations) account for more than 57% of cases. In the total population we found 15 patients compound heterozygotes or homozygotes (frequency of 8%). The mutation rate in pediatric population (minor than 16 years) was 85% (34/40 patients).

Conclusions. In agreement with the presence of genomic clusters of LDLR mutations, we identified the 6 mutations with the highest frequency in Southern Italy. In order to prevent fatal cardiovascular events, particular attention should be taken in the identification of compound heterozygous, homozygous and pediatric patients which represent a high percentage of FH population.

Reference

GENE EXPRESSION AND MICRONRNA SIGNATURE IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF METABOLIC PATIENTS

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Background. Metabolic syndrome (MS) is a cluster of risk factors (dysglycemia, hypertension, dyslipidemia, central adiposity) leading to increased cardiovascular risk (CVR) and diabetes. In order to identify putative novel biomarkers of MS onset and progression we studied whole-genome gene and miRNA profile of peripheral blood mononuclear cells (PBMCs) in patients with MS.

Materials and Methods. We enrolled 20 healthy controls (10F:10M) and 20 naïve patients (10F:10M; >3 ATPII criteria for MS) without organ damage. We collected clinical and biochemical data, and blood samples for PBMCs isolation and the study of gene and miRNA expression profiling (Illumina iScan).

Results. When compared to healthy controls, MS patients were characterized by increased BMI, abdominal circumference, blood pressure, insulin-resistance, glycaemia, and CVR, while HDLc was decreased (p<0.05). Up to 8000 genes were expressed in the PBMC transcriptome, with 68 significantly up-regulated and 44 down-regulated genes in MS group; of the 622 miRNAs
expressed in the PBMCs, 12 were significantly up-regulated and 2 were down-regulated in the MS group (cut-off 1.3 folds; adjusted p-value <0.001). With the Ingenuity Pathway Analysis, we dissected the biological networks differentially expressed in MS, and we found a strong down-regulation of lipid metabolism pathway (12 genes) and an up-regulation of cell cycle, cellular growth and proliferation (30 genes). The most intriguing gene was the ATP-binding cassette transporter A1 (ABCA1), a key player of cholesterol efflux from macrophages governing reverse cholesterol transport, which was down-regulated, positively correlated to HDLc, and negatively to BMI and CVR. Furthermore, we found other intriguing correlations between miRNAs, genes, and clinical variables.

Conclusions. PBMCs are active players in MS pathophysiology and their transcriptome is a source of putative biomarkers of MS diagnosis and progression, and targets for the management of lipid disorders and atherosclerosis.

EFFECTS OF ADD-ON ALISKIREN THERAPY ON PLAQUE INSTABILITY MARKERS IN HYPERTENSIVE ELDERLY PATIENTS UNDERGOING CAROTID ENDOARTERECTOMY

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Introduction. Hypertension stimulates atherogenesis and plaque instability. Many patients undergoing carotid endarterectomy (CEA) do not have BP to target despite drug therapy. Matrix metalloproteinases (MMPs) have been suggested to be involved in plaque instability whereas the tissue inhibitor of MMP (TIMP) was related to plaque stabilization and it is increased in calcified areas of carotid plaques. The aim of the present study was to analyze if add-on anti-hypertensive therapy with aliskiren or lercanidipine was able to affect plaque expression of MMPs, TIMP as well as CDKN2A, VEGF, and resistin, that are involved respectively in progression, destabilization and formation of plaques.

Materials and Methods. 52 hypertensive patients treated with two drugs and with cardiovascular co-morbidities (AIFA criteria for aliskiren online monitoring) enlisted for CEA were selected. Patients were assigned to one of three groups: group A hypertensive patients with BP under optimal control (n=27, control group), group B hypertensive with aliskiren as add-on therapy (n=12), and group C where the add-on was lercanidipine (n=13). All patients were undergoing surgery after two months of add-on therapy and carotid plaques were collected for analysis. Expression of MMP-1, -2, -7, -9, -12, TIMP-1, CDKN2A, VEGF, and resistin was analyzed by RealTime PCR/TaqMan assay.

Results. Patients treated with add-on drug therapy had BP <140/80 mmHg both in group B (aliskiren) and in group C (lercanidipine). Aliskiren group showed a reduction in the expression of almost all genes assayed compared to group C treated with lercanidipine as add-on, reaching significance for MMP-1, MMP-7 and MMP-9 (p<0.05). Both group B and C showed statistical reduction (p<0.05) for MMP-12.

Conclusions. Showed that aliskiren as add-on therapy was more effective than lercanidipine in reducing the expression of MMPs. In particular, the main MMPs responsible for atherosclerotic plaques progression, destabilization and rupture seem to be more potently downregulated by aliskiren add-on therapy. These results suggest a new therapy for patients with hypertension and vascular disease.

PLATELETS ACTIVATION IN PATIENTS WITH CLAUDICATIO INTERMITTENS: EFFECTS OF PHYSICAL TRAINING ON AT REST AND AFTER INDUCTION OF ISCHEMIA

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Background. Training is a documented effective treatment in patients affected from peripheral arterial disease. Platelet activation plays a pivotal role in atherosclerosis progression and cardiovascular events. In ischemic heart disease, platelet activation is reduced by aerobic training, while strenuous exercise is associated with enhanced activation. Few data can be found for patients with peripheral arterial disease on training. We aimed to evaluate the effects of aerobic training on platelet activation and oxidative stress at rest and after maximal walking exercise.

Methods and Results. We enrolled 27 patients with intermittent claudication. They were submitted to a 15 days aerobic training period (cycling and treadmill exercise under maximal walking capacity). Platelet function (PAF 100 with ADP and epinephrine, P-selectin) and oxidative stress (malondyaldehyde) were analyzed at rest and after maximal treadmill test, at the beginning and at the end of the period. At the end of training, absolute walking distance increased (450±180 vs 250±108 m; p<0.05), malondyaldehyde decreased (124±20 vs 147±25 ug/l; p<0.05), P-selectin decreased (0.81±0.31 vs 1.40±0.62 p<0.005), epinephrine platelet activation improved. Maximal treadmill test before training elicited significant ADP platelet activation, while the maximum treadmill test reduced platelets ADP activation at the end of training; the test caused increase in malondyaldehyde concentration at the beginning and at the end of protocol.

Conclusions. Training can cause an improvement of endothelial function and an increased release of NO and prostacyclin, these substances exert favorable effects on platelets function. Furthermore, ischemia-reperfusion, elicited by maximal treadmill test, increases the release of ADP that is inversely reduced after training. Aerobic supervised training in patients with peripheral arterial disease improves platelets aggregation, oxidative stress and platelets aggregation during maximal exercise. These data help explaining the benefit of training in PAD and atherosclerosis.
**MicroRNA PROFILING REVEALS NEW POTENTIAL MODULATORS OF INSULIN-RESISTANCE IN TYPE 2 DIABETES**

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**Background.** Type 2 Diabetes (T2DM) is a chronic disease characterized by an inadequate beta-cell response to the progressive insulin resistance. The tiny mechanism(s) underlying insulin-resistance and increased atherosclerosis burden in T2DM patients are not fully understood. MicroRNAs (miRNAs) are short (20-22 nucleotides of length), endogenous, non-coding RNAs representing a new class of regulators of gene expression. Remarkably, they are found in all cell type and their presence is clearly detectable in peripheral blood. Currently miRNAs involvement has been demonstrated in many diseases such as cancer, inflammatory and cardiovascular diseases. However, the role of miRNAs in T2DM in humans is not fully elucidated, thus aim of this study was to investigate the plasma miRNAs profile of diabetic patients.

**Materials and Methods.** Blood samples were collected from 11 diabetic patients and 11 matched control patients. T2DM diagnosis was formulated according currently available American Diabetes Association guidelines. We enrolled only drug-naïve patients before starting specific treatment. Patients with active inflammatory diseases, chronic kidney disease and cancer were excluded.

RNA was extracted according with previously validated methods, quantified and pooled and a wide microRNA expression profiling was performed (miRNome). Then, some of the miRNAs that were differently expressed between two groups were validated by RealTime-PCR (RT-PCR). Data analyses were performed with deltadelta Ct method. Finally, bioinformatics was used in order to identify the potential targets of these miRNAs.

**Results.** Microarray analysis showed that 4 miRNAs were upregulated whereas 21 miRNAs were downregulated in diabetic patients. Interestingly, RT-PCR validation confirmed a significant downregulation of let-7a (p=0.023) and let-7i (p=0.049). Moreover, we found a significant upregulation of miR-326 (p=0.006). Furthermore, an interesting trend supporting down-regulation of miR-16, miR-21 and let-7g in diabetic patients was found, despite these values did not reach statistical significance probably due the small study population. In silico analysis confirmed that the predicted targets of these miRNAs are able to modulate genes involved in insulin-sensitivity, including Adiponectin and IGF-1 receptor.

**Conclusion.** This study demonstrated that diabetes is associated with a modulation of the expression of plasma miRNA that are involved in insulin resistance. If confirmed, these findings will contribute to improve our knowledge on diabetes pathophysiology and lead to the identification of new innovative therapeutic approach.

**ORAL ADMINISTRATION OF LACTOBACILLUS ACIDOPHILUS, BIFIDOBACTERIUM INFANTIS AND STREPTOCOCCUS THERMOPHILUS INDUCES HEPATIC BILE ACID SYNTHESIS IN MICE**

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The gastrointestinal tract is homing a highly dynamic microbial ecosystem that has metabolic and protective functions. Gut microbiota is crucial for bile acid (BA) biotransformation thus modulating BA pool composition. The aim of the study was to characterize the effects of probiotic-mediated modification of gut microbiota in regard to BA metabolism.

Mice were administered vehicle or probiotic mixture VSL#3 for 21 days. Fecal probiotic DNA composition and bacterial enzyme gene expression and activity were weekly monitored while serum, biliary, fecal BA levels and pool size were measured at the end of the treatment. A metabolic flux study by using (3H)-taurocholic acid was performed to monitor ileal BA absorption. Finally, characterization of the bacterial lineages (phyla) present in the fecal microbiota was obtained by metagenomic sequencing and analysis. VSL#3 modified fecal bacteria DNA composition and increased the expression and activity of BA-deconjugating enzymes along with a reduced fecal conjugated/unconjugated BA ratio. VSL#3-treated animals exhibited enhanced fecal BA excretion that was associated with changes in ileal BA absorption and compensated by an increased hepatic BA neo-synthesis due to repression of the entero-hepatic farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis. Serum, biliary and BA pool size were unchanged upon VSL#3 treatment. When probiotic mixture was given to FXR-deficient animals, hepatic BA neo-synthesis appeared unchanged. Probiotic modification of gut microbiota is able to change BA metabolism via enhanced fecal excretion and hepatic neo-synthesis and may have a therapeutic potential in metabolic and inflammatory disorders of gut-liver axis where BA level modulation is desirable.

**T HELPER 17/T HELPER 1 INFLAMMATION IN ATHEROSCLEROSIS**

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A pathogenetic role for infections in atherosclerosis has been suggested by the detection of pathogens in the arterial vessels and by the association between atherosclerosis and serological responses to different pathogens, such as Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, herpes simplex virus, Haemophilus influenzae, or between the extent of atherosclerosis and the infectious burden.

Phospholipases are produced from bacterial pathogens causing very different diseases. One of the most intriguing aspects of phos-
phospholipases is their potential to interfere with cellular signaling cascades and to modulate the host-immune response. We investigated the role of the innate and acquired immune responses elicited by Chlamydia pneumoniae phospholipase D (CpPLD) in the pathogenesis of atherosclerosis. We evaluated the cytokine and chemokine production induced by CpPLD in healthy donors’ monocytes and in vivo activated T cells specific for CpPLD that infiltrate atherosclerotic lesions of patients with C. pneumoniae antibodies (12 males and 12 females; mean age 68.9 years) with or without anti-C. pneumoniae antibodies. We also examined the helper function of CpPLD-specific T cells for monocyte matrix metalloproteinase (MMP)-9 and tissue factor (TF) production as well as the CpPLD-induced chemokine expression by human venular endothelial cells (HUVECs). We found that CpPLD is a TLR4 agonist able to induce the expression of interleukin (IL)-23, IL-6, IL-1beta, TGF-β, and CCL-20 in monocytes, as well as CXCL-9, CCL-20, CCL-4, ICAM-1, and VCAM-1 in HUVECs. In the lymphocytes infiltrates of human atherosclerotic lesions, we showed a significant (p<0.001) predominance of T cells producing interleukin-17, gamma-interferon and other cytokines. Plaque-derived T cells produce IL-17 in response to CpPLD. Moreover, CpPLD-specific T lymphocytes display helper function for monocyte MMP-9 and TF production. CpPLD promotes Th17/Th1 cell migration through the induction of chemokine secretion and adhesion molecule expression on endothelial cells. CpPLD is able to drive the expression of IL-23, IL-6, IL-1β, TGF-β, and CCL-20 by monocytes and to elicit a Th17/Th1 immune response that play a key role in the genesis of atherosclerosis, thus suggesting that Th17/Th1 cell pathways and CpPLD may represent novel therapeutic targets for the prevention and treatment of the disease.

STUDIO GENETICO MOLECOLARE IN DUE CASI DI ABETALIPOPORTEINEMIA

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Introduzione. L’abetalipoproteinemica (ABL) è un raro disordine recessivo caratterizzato da livelli molto bassi di colesterolo totale, VLDL, LDL e chilomicroni, e la completa assenza di apolipoproteine B (apoB) nel plasma. L’ABL si presenta con ritardo di crescita, malassorbimento intestinale di lipidi, steatorrea, accumulo di lipidi nel fegato e negli entereociti. ABL è dovuta a mutazioni del gene MTP che codifica per una proteina microsomiale di trasformazione del gene già riscontrata nella popolazione Ashkenazita ed ha confermato la presenza di una mutazione iniziale di un codon di stop prematuro e causa la formazione di una proteina tronca di 372 amminoacidi priva di funzione.

Il paziente #WI è risultato appartenere alla comunità ebraica Ashkenazita, popolazione in cui è frequente (1:131) la presenza in eterozigosità di una sostituzione nucleotidica (c.2593 G>T) nell’esone 18 del gene MTTP risultante in una mutazione nonnonsense (p.G865X). Per tale motivo è stata eseguita nel paziente la ricerca di questa mutazione. L’analisi di sequenza ha rilevato la presenza allo stato omozigote della mutazione c.2593 G>T, che porta alla formazione di un codon di stop prematuro e causa la formazione di una proteina tronca di 864 aminoacidi.

Conclusioni. Questo studio ha confermato la presenza di una mutazione MTP già riscontrata nella popolazione Ashkenazita ed ha identificato una nuova mutazione di questa proteina, come causa di ABL.

DECREASED EXPRESSION OF ADIPONECTIN RECEPTORS IN HUMAN CAROTID Atherosclerotic Plaques

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Introduction. Adiponectin is a protein secreted by the adipose tissue with pleiotropic effects on metabolism. Its antiatherogenic action consists in the improvement of endothelial function and anti-inflammatory status in arterial walls (1). Adiponectin effects are mediated by two receptors, AdipoR1 and AdipoR2, which are ubiquitously expressed (2). The adiponectin resistance was demonstrated in obesity. We aim to verify if adiponectin-resistance occurs in human atherosclerotic plaques.

Materials and Methods. RNA from 49 carotid atherosclerotic plaques (advanced lesions), their respective adjacent regions (with a low grade lesions) and 7 healthy arteries (iliac and mesenteric) was extracted, reverse-transcribed and used for real-time PCR by specific TaqMan assays (Applied Biosystems). In vitro experiments for gene regulation studies were performed on primary Human Aortic Endothelial Cells and Smooth Muscle Cells (Lonza) co-cultured with the macrophage cell line THP-1.

Results. Expression levels of AdipoR1 were lower in advanced plaques as well as in their respective adjacent regions than in healthy arteries (p=0.020 and p=0.001 respectively). AdipoR2 levels gradually decreased from healthy arteries to plaque adjacent regions and to advanced plaques (all comparisons p<0.0001). In both cell types, expression of AdipoR1 was not affected by the coculture with THP-1. The presence of THP-1 decreased AdipoR2 expression in Smooth Muscle Cells (p=0.040), whereas a not statistically significant decrease was observed in Endothelial Cells.

Conclusion. We demonstrated a decreased expression of adiponectin receptors in initial and advanced plaques, suggesting a mechanism of adiponectin-resistance during atherosclerotic process. A local adiponectin-resistance could be a contributory cause of the lack of atheroprotective effects mediated by adiponectin. Presence of macrophages could be a contributory cause of AdipoR2 decrease.

References
LOW-GRADE INFLAMMATION MODULATES T-LYMPHOCYTE RENIN-ANGIOTENSIN SYSTEM ACTIVATION IN HYPERTENSIVES AND OBESES

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Purpose. Human T-lymphocytes were shown to be endowed with a functional active renin-angiotensin system (RAS) and to have a role in the development of hypertensive target organ damage. T-lymphocyte RAS seems to be activated in hypertensive patients with low-grade inflammation. Low-grade inflammation is reported to mediate cardiovascular risk also in obeses. The aim of this study is to assess the activation of T-lymphocytes RAS in hypertensives and/or obeses and the possible correlation with low-grade inflammation.

Methods. T-lymphocytes were obtained from peripheral blood samples of 8 obeses (BMI>29) (7M, 1F, 47±11 years), 9 hypertensives with BMI<29 (7M, 2F, 63±11 years) and 7 hypertensives with BMI>29 (7M, 52±10 years). No patient was in therapy with ACE-inhibitors and/or Angiotensin receptor blockers. Seven healthy subjects formed the age and sex-matched control group. After isolation, T-lymphocytes were put in culture and at 6 hours mRNA for ACE was quantified by RT-PCR. Presence of low-grade inflammation was defined by serum levels hsCRP >2 mg/L.

Results. hsCRP showed a large distribution in groups, with mean values significantly higher than controls. All hypertensives with BMI>29 presented hsCRP levels >2 mg/L. ACE mRNA levels showed a large distribution inside the three groups as well, with mean values significantly higher than controls. ACE mRNA levels were linearly related to hsCRP levels (R=0.79; p<0.0001). There was a positive correlation between hsCRP levels and BMI. No significant correlation was found between ACE mRNA levels and BMI. In the three groups, ACE mRNA levels were significantly higher than controls only in patients with low-grade inflammation.

Conclusion. Circulating T-cells ACE gene expression is modulated in presence of low-grade inflammation. In hypertensive and/or obese patients, a selective T-lymphocytes RAS activation can occur. If these results will be confirmed, T-cells RAS activation could be considered as a new marker for the optimization of both cardiovascular risk definition and antihypertensive therapy management.

LIRAGLUTIDE IS MORE EFFECTIVE THAN EXENDIN-4 AS A POSTCONDITIONING AGENT IN LIMITING REPERFUSION INJURY IN BOTH WKY AND SHR-SP RATS WITH LEFT VENTRICULAR HYPERTROPHY

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Introduction. Exendin-4 (ex4) postconditioning has been shown to limit reperfusion injury (RI) in experimental and clinical set-

ings. Left ventricle hypertrophy (LVH) may be associated with increased RI. Our objective was to study ex4 and liraglutide post-conditioning (PostC) in hearts with LVH, isolated from hypertensive SHR-SP (hypertensive LVH) rats.

Methods. Hearts isolated from WKY (control) and SHR-SP rats (11-15 weeks old) were subjected to 35 min LAD occlusion-2 hrs reperfusion, with ex4 0.3 nM or liraglutide 0.3 nM present during the first 15 min in treated hearts. Evans blue/TTC method was used to determine area-at-risk (AAR) and infarct size (% of AAR). Akt phosphorylation (Akt-P) was measured on western blots after 3 min of reperfusion. Arterial blood pressure (BP) was measured in conscious animals by tail cuff method.

Results. BP and heart/body weight ratio were increased in SHR-SP compared to WKY rats (p<0.0001 for both parameters). Infarcts were larger in SHR-SP than in WKY (65.7 +/-3.2, N=7 vs 37.1 +/-3.4, N=12 respectively; P<0.05). Exe-4 and liraglutide Postc decreased infarct size (IS) after 35 min ischemia in WKY (p<0.05). Liraglutide and preconditioning, but not Exe-4, decreased IS after 35 min in SHR-SP (p<0.05). Exe4 Postc decreased IS after 15 min ischemia in SHR-SP (p<0.05). In WKY hearts, ex4 treatment significantly decreased diastolic contracture and increased left ventricle developed pressure. Liraglutide, but not ex4, decreased diastolic pressure in SHR hearts. Degree of Akt phosphorylation was smaller in LVH hearts compared to normal hearts.

These data suggest that 0.3 nM liraglutide was more effective than 0.3 nM ex4 in limiting reperfusion injury in both WKY and SHR-SP. In both WKY and SHR-SP hearts there was a loss of response to PostC by ex4 with increasing ischemia time and infarct size. This loss of response to PostC occurs earlier in hypertrophy hearts.

SERUM CHOLESTEROL EFFLUX CAPACITY INVERSELY CORRELATES WITH ARTERIAL STIFFNESS IN HEALTHY SUBJECTS

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Objective. Little is known on the impact of HDL athero-protective properties on vascular remodeling in healthy subjects. We investigated the relationship between serum cholesterol efflux capacity (CEC), an indicator of HDL functionality, and Pulse Wave Velocity (PWV), an indicator of arterial stiffness, in healthy subjects.

Methods and Results. 167 healthy subjects (54 males, 113 females) underwent serum CEC measurement (aqueous diffusion and ATP-binding cassette A1 (ABCA1)-dependent cholesterol efflux) and carotid-femoral PWV with a high-fidelity tonometer. Both ABCA1-mediated CEC and PWV did not correlate with HDL-C levels, either as a whole group and as males and females separately. In an unadjusted model, PWV inversely correlated with ABCA1-dependent cholesterol efflux (r=-0.183, p=0.018). No correlation was found between PWV and aqueous diffusion-dependent CEC (r=0.129, p=0.095). In a nested linear regression model, controlling for age, sex, body mass index, mean arterial pressure, serum low-density lipoprotein, HDL and glycated hemoglobin, PWV displays a significant negative regression on ABCA1-dependent CEC (β = -0.204, 95%CI -0.371/-0.037). The finding that ABCA1-dependent CEC, but not serum HDL cholesterol level, is a significant
Development in apoE-/- mice. The effect of compound 2 on spontaneous atherosclerotic plaque development in cultured human umbilical endothelial cells (HUVEC) indicating a small molecule Rac inhibitor capable to interfere with SMC migration and to test in in-vitro and in-vivo models of atherosclerosis. Compound 2 profoundly affected cytoskeleton organization of cultured human SMCs and inhibited SMC migration in response to PDGF-BB in a concentration dependent manner with an IC50 value of 5.8 µM. The IC50s for Rac inhibition of these compounds were between 2.9 and 29.1 µM and similar potencies were observed in all patients affected by polygenic hypercholesterolemia after three and six weeks of treatment with Sterolip (a new nutriceutical product in bag containing 1.6 g of plant sterol) with respect to a placebo; secondary objectives were to verify the tolerability of the product, to test the long term efficacy after 12 weeks of treatment, to evaluate the response to the therapy in relation with three genetic variants involved in sterol absorption.

Methods. We enrolled 60 patients over 18 years of age, affected by polygenic hypercholesterolemia in primary prevention with cardiovascular risk <20% in the next ten years. 30 patients were double-blind randomized with placebo and 30 patients with Sterolip. The double blind treatment lasted six weeks at the end of which all patients were treated with Sterolip in open-label for other six weeks. Lipid profile was determined before treatment and every three weeks. We genotyped all patients for APOE, NPC1L1 c. 816C>G (L272L) and ABCG8 D19H polymorphisms.

Results. The parameters that in the treatment group were significantly reduced compared to the placebo group were total cholesterol (TC) and LDL Cholesterol (LDL-C) at three weeks of treatment (p<0.05). The product was good tolerated. Long term therapy is efficacious only in a few cases with a large interindividual variability. Carriers of G allele of NPC1L1 polymorphism have demonstrated a significant more consistent reduction of TC after three and six weeks of treatment in comparison to C allele carriers (p<0.05). The product was good tolerated. Long term therapy is efficacious only in a few cases with a large interindivid-ual variability. Carriers of G allele of NPC1L1 polymorphism have demonstrated a significant more consistent reduction of TC after three and six weeks of treatment in comparison to C allele carriers (p<0.05).

Conclusions. In accordance with data published in literature this study demonstrated a clinically significant action of plant sterols after three weeks of treatment. In genetically predisposed indi-viduals, the therapy may be considered for a more time.

Evaluation of Efficacy of a New Nutriceutical Product Containing Plant Sterols in Bag (Sterolip ESI) in Reducing Cholesterol Levels in Patients with Polygenic Hypercholesterolemia

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Primary objective was to establish how lipid profile is modified in patients affected by polygenic hypercholesterolemia after three and six weeks of therapy with Sterolip (a new nutriceutical product in bag containing 1.6 g of plant sterol) with respect to a placebo; secondary objectives were to verify the tolerability of the product, to test the long term efficacy after 12 weeks of treatment, to evaluate the response to the therapy in relation with three genetic variants involved in sterol absorption.

Methods. We enrolled 60 patients over 18 years of age, affected by polygenic hypercholesterolemia in primary prevention with cardiovascular risk <20% in the next ten years. 30 patients were double-blind randomized with placebo and 30 patients with Sterolip. The double blind treatment lasted six weeks at the end of which all patients were treated with Sterolip in open-label for other six weeks. Lipid profile was determined before treatment and every three weeks. We genotyped all patients for APOE, NPC1L1 c. 816C>G (L272L) and ABCG8 D19H polymorphisms.

Results. The parameters that in the treatment group were significantly reduced compared to the placebo group were total cholesterol (TC) and LDL Cholesterol (LDL-C) at three weeks of treatment (p<0.05). The product was good tolerated. Long term therapy is efficacious only in a few cases with a large interindividual variability. Carriers of G allele of NPC1L1 polymorphism have demonstrated a significant more consistent reduction of TC after three and six weeks of treatment in comparison to C allele carriers (p<0.05).

Conclusions. In accordance with data published in literature this study demonstrated a clinically significant action of plant sterols after three weeks of treatment. In genetically predisposed individuals, the therapy may be considered for a more time.

Incidence and Severity of Nonalcoholic Fatty Liver Disease Associated with Impaired Lipid and Glucose Metabolism Are Predicted by SREBF Polymorphism

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Background. Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease, encompasses a histological spectrum, ranging from simple steatosis (SS) to steatosis plus necro-inflammation (nonalcoholic steatohepatitis, NASH), which can be differentiated only by liver biopsy. While SS has a benign hepatological course, NASH can progress to end-stage liver disease. Sterol regulatory element-binding factor (SREBF) genes code for key predictor of PWV in healthy subjects points to the relevance of HDL function in vascular physiology and arterial stiffness prevention along life.

Development of New Rac1 Inhibitors as Potential Pharmacological Agents for the Treatment of Atherosclerosis

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Experimental and clinical observations have documented the role of the small GTPase Rac1 protein and cardiovascular diseases opening to the development of new potential pharmacological intervention. In this study we characterized a new class of selective small molecule Rac inhibitors with the 3-aryl-1H-pyrazole-5-carboxamide nucleus. Starting from our previous identification of Rac inhibitors (1), through a computational approach, 57 chemical entities were identified and their Rac inhibitory efficacy evaluated by G-LISA assay. 23 compounds were found to reduce Rac-GTP levels in cultured cells by more than 24.8%. A comparative analysis at 25 GTP levels. The IC50s for Rac inhibition of these compounds were between 2.9 and 29.1 µM and similar potencies were observed in cultured human umbilical endothelial cells (HUVEC) indicating a small molecule Rac inhibitor capable to interfere with SMC migration and to test in in-vitro and in-vivo models of atherosclerosis. Compound 2 profoundly affected cytoskeleton organization of cultured human SMCs and inhibited SMC migration in response to PDGF-BB in a concentration dependent manner with an IC50 value of 5.8 µM. More interestingly, incubation of human monocytic cell line THP-1 with Compound 2 (10 µM) completely abrogated their adhesion to RhoA protein, while compound 1 partially reduced RhoA-GTP levels. The IC50 for Rac inhibition of these compounds were between 2.9 and 29.1 µM and similar potencies were observed in cultured cells by more than 24.8%. A comparative analysis at 25
nuclear transcription factors regulating lipid homeostasis: sterol regulatory element binding protein (SREBP)-1c regulates hepatic de novo lipogenesis and insulin sensitivity, while sterol regulatory element-binding factor-2 (SREBP-2) codes for SREBP-2, a master transcriptional regulator of genes involved in cellular cholesterol biosynthesis, uptake, and export. Therefore, SREBF-1c and SREBF-2 may modulate the genetic susceptibility to NAFLD and NASH.

**Objective.** Factors predisposing to non-alcoholic fatty liver disease (NAFLD) and associated cardio-metabolic disorders are unknown. Sterol regulatory element-binding protein (SREBP)-1c and SREBP-2, transcription factors regulating lipogenesis and cholesterol metabolism have been experimentally connected to NAFLD, but no human data exist. In population-based studies, single nucleotide polymorphisms (SNPs) in SREBF-1 gene have been connected to obesity, insulin resistance and T2DM, and the functional SNP rs133291 C/T in the SREBF2 gene has been linked to serum LDL-cholesterol, but there are no human data on the impact of these SNPs on the risk of developing NAFLD and associated metabolic abnormalities. We hypothesized SREBF-1c/2 SNPs may not only predispose not only to NAFLD/NASH, but also affect NAFLD-associated metabolic risk. We aimed at:

1) prospectively assessing the role of SREBF-1c/2 SNPs in promoting NAFLD development in apparently healthy subjects;

2) elucidating mechanisms connecting SREBF-1c/2 to liver injury, glucose and lipid homeostasis in established NAFLD.

**Methods.** We followed-up 165 non-obese nondiabetic participants in a population-based study, without NAFLD/metabolic syndrome, characterized for 2 common SREBF-1c and SREBF-2 polymorphisms, diabetes, physical activity, adipokines, CRP, and endothelial dysfunction markers. NAFLD developers underwent an OGTT with Minimal Model analysis of glucose homeostasis parameters, and an oral fat tolerance test with measurement of plasma lipoproteins, adipocytes, and hepatocyte apoptosis marker cytokeratin-18 fragments.

**Results.** After 7 years, 29% subjects developed NAFLD and 5% developed diabetes. SREBF-1c and SREBF-2 predicted incident NAFLD; SREBF-2 predicted non-alcoholic steatohepatitis (NASH), diabetes, CRP and endothelial dysfunction markers. In NAFLD patients, SREBF-1c predicted histological steatosis and hepatic insulin resistance, SREBF-2 predicted progressive liver histology, hepatic/muscle adipose insulin resistance, pancreatic β-cell dysfunction, and an altered fat tolerance: higher postprandial lipemia, cholesterol enrichment of triglyceride-rich lipoproteins and ox-LDLs, HDL-C reduction, adipokine imbalance (lower adiponectin and higher resistin), and higher cytokeratin-18 fragments.

**Conclusions.** SREBF-2 predisposes to NASH and cardio-metabolic disorders by affecting glucose homeostasis and dietary fat tolerance. We showed polymorphisms in genes coding for nuclear transcription factor SREBP-2 predict incidence and severity of NAFLD and associated abnormalities in glucose and lipid metabolism and in adipokine response to dietary fat. Our findings may have relevant implications: from a clinical standpoint, SREBF-1/2 SNPs may be used to select NAFLD patients at higher risk of developing progressive liver disease and cardio-metabolic complications for tight monitoring and experimental treatments. Furthermore, future research should verify whether a genetically-mediated maladaptive response to a chronic, daily, repetitive stress like fat ingestion may link chronic overfeeding to obesity and its complications and to assess whether SREBP-2 pathway modulation may prevent cholesterol lipotoxicity in different tissues more effectively than currently available strategies, which selectively target hepatic cholesterol synthesis or intestinal absorption.

**SMALL DENSE LDL PROFILES IN FAMILIAL COMBINED HYPERLIPIDEMIA AND IN FAMILIAL HYPERCHOLESTEROLEMIA**

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**Introduction.** Differences between small dense LDL (sd-LDL) profiles were evaluated in 108 probands with Familial Combined Hyperlipidemia (FCHL), 117 probands with Familial Hypercholesterolemia (FH) and 146 normolipidemic, normotensive, normoglycemic healthy subjects among those consecutively admitted to the outpatient Lipid Clinic of the “Federico II” University of Naples.

**Methods.** LDL particle separation was performed by Lipoprint System: 7 LDL subfractions (LDL1 to LDL7) were obtained, mean LDL particle size and LDL score (% of sd-LDL) were calculated.

**Results.** LDL score was significantly (p<0.001) higher in FCHL patients as compared to FH patients (21.6±1.3 vs 15.3±1.0 mg/dL) and controls (3.3±5.0 mg/dL); mean LDL size was significantly lower (p<0.001) in FCHL patients than FH patients (262.8±0.5 vs 264. ±0.4 Å) and controls (271.1±2.8 mg/dL). In a subsequent analysis we compared the amount of cholesterol in each LDL subfractions of the two groups of patients and controls. LDL I and LDL II cholesterol were significantly (p<0.001) higher in FCHL patients as compared to FCHL patients (69.0±1.9 vs 44.4±1.9 mg/dL for LDL I) and controls (47.1±1.5 mg/dL). (69.5±2.0 vs 42.8±1.5 vs 19.1±8.6 mg/dL for LDL II) (FH vs FCHL vs controls).

**Conclusions.** These findings indicate that FCHL patients have LDL score significantly higher and mean LDL size significantly lower than FH patients and healthy subjects. Small, dense LDL particles have been associated with an increased risk of coronary artery disease. More studies are envisaged to investigate the specific contribution of LDL subfractions to cardiovascular risk in FH patient.

**THE IMPROVED BIOCHEMICAL DIAGNOSTICS OF THE LIPID PROFILES IN THE FRAMEWORK OF REGIONAL NETWORK FOR INHERITED LIPID DISORDERS: FIRST REPORT**

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Familial combined hyperlipidemia (FCHL) is a severe inherited hyperlipidemia with a high cardiovascular mortality. Affected individuals have elevated cholesterol or triglyceride concentrations or both. Such a lipid profile is frequently associated with an unfavourable decrease in high density lipoprotein concentration, an elevated apolipoprotein B and an increased prevalence of atherogenic, small, dense low-density lipoprotein (sd-LDL) subfractions. Family studies are necessary to establish the diagnosis of FCHL in each patient.

Since it is not always possible to get some biochemical data from...
first-degree relatives, the dosage of small dense LDL can be performed in these cases. LDL particles separation is performed by Lipoprint System. The proportion of sd-LDL particles to the whole LDL area is calculated (LDL score). An LDL score higher than 10.0 mg/dL is related in multivariate analysis to FCHL diagnosis, sensitivity 78% and specificity 89%. (Atherosclerosis 2009).

In our dataset we screened 139 patients with possible FCHL but without biochemical data from first-degree relatives; 67 patients (48%) had LDL score >10 mg/dL and FCHL diagnosis of probable FCHL was done.

The improved biochemical diagnostics of the lipid profiles which include the dosage of the LDL sub-fractions has a precise organizational importance regarding the appropriateness of the prescription. In fact, it allows to value, in absence of a familiar history, whether a drug can be refundable or not with a clear feedback and efficacy on the appropriateness of the A.O.U Federico II and on the regional induced cost.

**CIGARETTE SMOKE CONDENSATE MODULATES THE EXPRESSION OF METALLOPROTEINASES AND CYTOKINES BY HUMAN MONOCYTES AND MACROPHAGES**

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Cigarette smoke (CS) is a leading risk factor for atherosclerosis. Despite a growing body of literature describing the effects of CS, the inflammatory pathways involved in smoke-induced responses are still unclear since CS may both activate and/or inhibit inflammatory gene expression. In our study, we investigated the effects of CS condensate (total particle matter, TPM) on the expression of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines in human monocytes/macrophages. Human monocytes (HM) were isolated from peripheral blood of healthy non-smoker volunteers by Ficoll-Paque technique; HM-derived macrophages (HMDM) were obtained following HM differentiation. Cells were treated with increasing concentrations of TPM (4-62 µg/ml) for 24 hours: gene expression was analyzed by RT-PCR, and protein expression by ELISA or Western Blotting. In HMDM, TPM stimulated, in a concentration-dependent and statistically significant manner, MMP1 gene expression up to 6-fold, while it did not show any significant effect on the expression of MMP9 or TIMP1, the physiological inhibitor of both MMPs. TPM increased MMP1 expression, both at the mRNA and protein level (by 20- and 5-fold, respectively) also in HM. Moreover, TPM increased, in a concentration-dependent manner, IL1beta, IL8, TNF-alpha and TLR-4 expression. The TLR-4 pathway has been shown to regulate some CS effects on gene expression. The pretreatment with PDTC, a well-known NFkB inhibitor, completely abolished TPM stimulatory effect on IL8 and TNF-alpha, suggesting a role for NFkB signaling in cytokines up-regulation, possibly through a TLR-4 mediated pathway. These results indicate that TPM might affect the atherogenic process by increasing the collagenolytic and pro-inflammatory potential both in HM and HMDM. The mechanism(s) by which TPM can alter gene expression of cytokines and MMPs need to be further investigated; at the present our preliminary data suggest that NFkB signaling seems to be involved.

Study funded by British American Tobacco, Southampton, UK.

**REPLICATION OF LIPOPROTEIN RECEPTOR RELATED PROTEIN 1, TUDOR DOMAIN-CONTAINING PROTEIN 10 AND SOLUTE CARRIER FAMILY 30 MEMBER 8 GENE VARIANTS IN ABDOMINAL AORTIC ANEURYSM**

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Abdominal aortic aneurysm (AAA) has a multifactorial etiology and the relevance of genetic factors is getting increasing interest. A recent GWAS demonstrated an association between rs1466535 low density lipoprotein receptor related protein 1 (LRP1) polymorphism and AAA. Moreover, two other polymorphisms, rs6674171 in TDRD10 and rs3019885 in SLC30A8, showed conflicting associations. Aim of this study was to perform a GWAS data replication in an Italian cohort of AAA patients (n=423) and age and gender comparable healthy subjects (n=423).

Genotype distribution was significantly different between patients and controls for LRP1 and SLC30A8 polymorphisms. Carriers of SLC30A8 G allele showed a decreased AAA susceptibility, whereas carriers of LRP1 T allele were at increase risk for AAA. In the univariable logistic regression analysis the polymorphisms showed the following odds ratios (OR): rs3019885 SLC30A8 OR=0.73 (95%CI 0.54-0.98) p=0.042, and rs1466535 LRP1 OR=1.65 (95%CI 1.24-2.20) p=0.001. In a multivariable logistic regression analysis adjusted for traditional cardiovascular risk factors and chronic obstructive pulmonary disease only LRP1 polymorphism remained a significant and independent risk factor for AAA: OR=1.85 (95%CI 1.20-2.84), p<0.01. Our results confirmed the association of LRP1 gene with AAA, but while the GWAS study showed that the risk allele was C, in our study the risk allele was T. This discrepancy might be due to the wide difference in age of AAA patients and controls in the GWAS (median age 72 in AAA vs 45 and 52 in UK blood donors and Birth Cohort subjects). On the other hand, our data have a higher biological plausibility. In fact, in previous studies Lrp1 knock-out mice showed aortic dilatation, atherosclerosis and aneurysm, and the T allele was associated with LRP1 reduced expression.

In conclusion, our study confirms and adds new information on the role of rs1466535 polymorphism and LRP1 gene in AAA susceptibility and pathogenesis.

**OFF-TARGET EFFECTS OF THROMBOLYTIC DRUGS: APOLIPOPROTEIN A-I PROTEOLYSIS BY ALTEPLASE AND TENECTEPLASE**

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Background and aim. The administration of thrombolytic drugs is of proven benefit in a variety of clinical conditions requiring...
EVALUATION OF CIRCULATING PROGENITOR CELLS IN AN ELDERLY POPULATION: THE MUGELLO STUDY

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Background. The precise mechanisms underlying the relationship between increasing age and increased risk for vascular diseases are not completely elucidated. Circulating progenitor cells (CPC) and endothelial progenitor cells (EPCs), play a role in the development and progression of atherosclerosis. Clinical studies have demonstrated in young and middle-aged population that EPCs inversely correlated with the presence of traditional cardiovascular risk factors and with circulating pro-inflammatory molecules, but scarce data in the elderly are available. We used data from the Mugello Study, a prospective study which enrolled ultranongenarian subjects, who lived in the Mugello Area to evaluate the relationship between CPC, CPC, CEC and advanced age.

Methods. Circulating CEC, CPCs and EPCs were assessed by flow cytometric analysis and were defined as CD146+/CD31+/CD133 (CEC), CD34+, CD133+, CD34+/CD133+ (CPC) and CD34+/KDR+, CD133+/KDR+, CD34+/CD133+/KDR+ (EPC) respectively.

Results. We enrolled 270 ultranongeranarians (198F/72M) with a median age of 92(90-103) years. Ultranongeranians showed a significantly higher number of CEC(CD34+40) cells/106 events vs 336(359-414) cells/106 events; CD133+: 314(30-1030) vs 321(246-390) cells/106 events; CD34+/CD133+307 (30-1010) vs 294(224-364) cells/106 events. In the Mugello population CEC, CPC and EPC number was not affected by traditional cardiovascular risk factors. Furthermore we found a significant relationship between CPC and EPC and leukocyte number (CPC: CD34+: r=0.162, CD133+: r=0.165, p=0.005; CD34+/CD133+: r=0.190, p=0.01; CD34+/KDR+: r=0.142; CD133+/KDR+: r=0.148; CD34+/CD133+/KDR+: r=0.144, p<0.05 respectively).

Conclusions. Our results demonstrate the presence of an endothelial dysfunction, as documented by high CEC and low EPC number in ultranongeranians. Moreover the negative relationship between CPC, EPC and leukocyte number suggests that an inflammatory state significantly affects the regenerative capacity in the elderly.

ABCg1 AND SR-BI ON ATHEROSCLEROSIS

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A natural mechanism to prevent excessive macrophage cholesterol accumulation in the arterial wall, that leads to development of atherosclerosis, is Reverse Cholesterol Transport (RCT). Numerous proteins are involved in RCT, including ABC-transporter G1 (ABCg1) and scavenger receptor Bl (SR-BI). Both ABCg1 and SR-BI promote cholesterol efflux to mature HDL, while SR-BI is also essential for the selective uptake of cholesteryl esters from HDL by the liver, the last step of RCT. The aim of this project is to verify the effects of combined ABCg1 and SR-BI deletion on HDL cholesterol metabolism and the development of atherosclerosis. Hereto, unique ABCg1/SR-BI double knockout (dKO) mice were generated and fed with a semi synthetic high cholesterol Western-type diet (WTD), containing 15% fat and 0.25% cholesterol. Upon challenge with WTD, SR-BI KO and dKO mice showed increased total cholesterol levels (345±72 mg/dl and 295±35 mg/dl respectively) compared to WT and ABCg1 KO mice (93±18 mg/dl and 133±28 mg/dl respectively). A slightly lower amount of VLDL and HDL was observed in dKO mice in comparison to single SR-BI KO, but this failed to reach statistical significance. Furthermore, both WT and ABCg1 KO mice did not develop atherosclerotic lesions development under these mild dietary conditions, while SR-BI deficiency induced atherosclerotic development. No added effect of combined deletion of ABCg1 and SR-BI on lesion size was observed in mice on WTD as compared to single deletion of SR-BI (171±8×103 mm2 vs. 108±33×103 mm2). A trend towards higher white blood cell counts was observed in blood of ABCg1/SR-BI dKO mice in comparison with WT and single ABCg1 and SR-BI KO animals. In conclusion we show for the first time that, despite the lower serum cholesterol levels, deletion of ABCg1 did not affect the atherosclerosis susceptibility of SR-BI KO mice, probably as a result of increased leukocytosis.
METABOLIC ANALYSIS OF PLASMA FROM ALZHEIMER DISEASE PATIENTS

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Alzheimer Disease is a degenerative disease characterized by progressive impairment of cognitive function. The main feature of AD is the generation of an abnormal peptide, beta amyloid 42 (Aβ42) from Amyloid Precursor Protein (APP). Aβ42 is the main constituent of neurotangles and amyloid plaques, microscopic lesions found in AD patients' brain. Aβ42 triggers an inflammatory response that is responsible for most of the observed tissue damage. The diagnosis of AD is a complex task, mostly based on imaging techniques and clinical evaluation of the patient's neurological and cognitive functions. The search for plasma biomarkers able to detect early mild cognitive impairment is one of the recent attempt to help the clinician with new diagnostic tools. In this study we focused on a gas-chromatography mass-spectrometry (GC-MS) analysis coupled to chemometric automated metabolomic analysis of AD plasma samples compared with plasma of healthy subjects of comparable age and gender. Sera from twenty AD and twenty controls have been subjects to a procedure optimized to extract short chain organic acids, sugars and some fatty acids that can be detected by GC coupled to ion trap/MS detection. The method allowed the detection of over five thousands of individual ions that have been collected and measured by the XCMS software. After automated peak detection and alignment by XCMS, peaks have been normalized by a set of internal standards (C13 Leucine, C13 palmitic acid) and clustered into putative compounds by a homemade software. About 80 compounds were differentially expressed between AD and controls. After manual verification of the automated data, most of the compounds have been excluded since they represent column leakage or method artifacts, but some compounds represent true plasma constituents that are under investigation. Current findings will be presented after putative compound identification by the AMDIS/NIST software.

TOLL-LIKE RECEPTOR 3 AND INTERLEUKINE1β EXPRESSION IN CIRCULATING PROGENITOR CELLS ISOLATED FROM PATIENTS WITH HYPERTENSION AND RHEUMATOID ARTHRITIS

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Background. Circulating progenitor cells (CPCs), including CD34+ cells and endothelial progenitor cells (EPCs), play a role in delaying atherosclerosis and cardiovascular disease. Toll-like receptor 3 (TLR3), a member of the pathogen recognition receptors that mediates immune response and activates inflammatory genes, is involved in the development of atherosclerosis. TLR3 has been recently detected in EPCs and its “in vitro” activation appears to induce cytokine expression and apoptosis. We measured the expression of TLR3 and Interleukine1β-mRNA in CPCs isolated from patients with high and low grade chronic inflammatory stimuli. The aim was to evaluate whether systemic inflammation is associated with CPC number and CPC expression of TLR3 and IL-1β.

Methods. CD34+ cells were isolated from peripheral blood of 41 untreated hypertensives with left ventricular hypertrophy and carotid atherosclerosis, from 21 untreated patients with rheumatoid arthritis (RA) and from 62 matched controls. TLR3 and IL-1β-mRNA expression were measured in enriched sample of CD34+ cells. Plasma C-reactive protein (CRP) and fibrinogen levels were also measured.

Results. With respect to controls, CD34+ cell number was higher in hypertensives and lower in RA. CRP and fibrinogen levels were higher in patients than in controls but the highest value were measured in RA. TLR3-mRNA expression was not changed in hypertensives with respect to controls while it was significantly higher in RA. IL-1β-mRNA expression was increased in both groups of patients but RA had highest value. CRP and fibrinogen levels were associated with IL-1β expression; moreover, the increased expression of IL-1β was associated with lowering CPC number.

Conclusions. The modifications induced by the low grade inflammation in hypertensives on TLR3 and IL-1β appear do not affect directly CPCs; the higher inflammatory grade in RA is associated to an increased expression of TLR3 and IL-1β in CPCs, which appear to influence CPCs reduction.

ABSORBED CHOLESTEROL AND HDL-C LEVELS DURING THE “ACUTE PHASE RESPONSE”

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Cholesterol absorption varies in different metabolic conditions: metabolic syndrome (MS) is characterized by low absorption/high synthesis and common hypercholesterolemia by high absorption/low synthesis. “Acute phase response” (APR), induced by infections, malignancy and autoimmunity, modifies lipid pattern, mainly by decreasing HDL-C through several mechanisms. Little is known whether the APR state modulates cholesterol homeostasis. To this purpose we studied cholesterol absorption and synthesis markers in 46 patients admitted to our Department for several diseases associated to APR: sepsis/septic shock and acute exacerbation of autoimmune/rheumatologic diseases. On an average of two-three days after the admittance we determined lathosterol, sitosterol and campesterol (gas chromatography/ mass spectrometry), total cholesterol, triglycerides, HDL-C, LDL-C and acute phase reactants. These parameters were also determined in 57 patients with MS and in 32 controls. HDL-C and LDL-C were lower in APR patients (28±11 mg/dl and 80±28 mg/dl respectively) compared to controls (51±19 mg/dl and 126±32 mg/dl, p<.001) and to MS (37±15 mg/dl and 156±49 mg/dl, p<.002). Log campesterol values were lower in APR (1.38±0.3 102 umol/mmol cholesterol) compared both to controls (1.6±0.37 102 umol/mmol cholesterol, p<.02) and to MS (1.55±0.26 102 umol/mmol cholesterol p.<.05); sitosterol was slightly lower in APR and lathosterol higher in MS, although not significantly. Regression analysis showed that the multivariate model including gender (β=358),
triglycerides (β−1.80) and campesterol (β−1.69) explained 20% of HDL-C variability (R<0.45, p<0.001), irrespective of confounders. Previous studies showed that the aliquot of absorbed cholesterol influences HDL formation, a process occurring also in the intestine; reduced campesterol levels in patients with an acute phase response could reflect both a reduced cholesterol intake and a reduced intestinal absorption. Our data are preliminary and more studies are needed for a better understanding the role of synthesis/absorption in the complex puzzle of lipid modifications during acute phase response.

**IN MORBID OBESITY A LOWER HOMA-INDEX IDENTIFIES METABOLICALLY HEALTHY SUBJECTS WITH NO EARLY VASCULAR IMPAIRMENT**

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Obesity is associated with atherosclerosis since its earliest stages. Part of obese subjects can be considered metabolically healthy and BMI alone is not usefulness for this distinction. The aim of our study was to compare the predicting role of HOMA index and of BMI on early vascular impairment in a population of morbidly obese subjects. We examined 65 morbidly obese subjects (BMI 44.6±7 Kg/m²). Glycemia, insulinemia, lipids, flow mediated dilatation (FMD), carotid intima media thickness (IMT) and visceral fat area (VFA) by ultrasound were performed in all subjects. The population was divided on the basis of HOMA-IR median values: >/>= 3.5 group 1, <3.5 group 2. Group 1 had significantly higher values of BMI (p<0.05), waist circumference (p<0.0001), VFA (p<0.0001), triglycerides (p<0.005), glyceremia (p<0.0001), and lower HDL-C (p<0.005) than group 2; FMD was significantly lower (p<0.05) and IMT significantly higher (p<0.05) than in the group 2. Dividing the same population on the basis of BMI median values those with higher BMI, although showing higher VFA and HOMA-IR had no significant differences in lipid parameters, FMD and IMT. At stepwise regression analysis VFA was the independent predictor of reduced FMD (beta −0.541, p 0.002). HOMA-IR (beta 0.399 p<0.001) was the independent predictor of IMT (beta 0.413, p<0.001). Twenty patients were re-evaluated after bariatric surgery: FMD significantly improved in both groups, with a better improvement in the group with pre-bariatric HOMA index <3.5. Although BMI above 30 kg/m² is associated with a reduced survival, its role seems far less helpful in the prediction of early atherosclerosis in morbidly obese. BMI, index of overall adiposity, does not discriminate the kind of fat distribution and subsequently the metabolic and vascular derangement; HOMA-IR, strictly related to visceral fat, is an expression of metabolic impairment thus able to predict early vascular damaging.

**ARTERIOPATIA OBLITERANTE DEGLI ARTI INFERIORI, IPOVITAMINOSI D E MARKERS DI RIMODELLAMENTO OSSEO**

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**Introduzione.** L’ipo vitaminosi D, molto comune nella popolazione anziana, favorisce l’osteoporosi ed aumenta il rischio cardiovascolare. Una correlazione inversa è stata rilevata tra calcificazioni coronariche, ispezzamento medio-intimale (IMT) carotide e livelli di vitamina D. Il presente studio ha avuto come scopo quello di valutare le eventuali correlazioni tra arteriopatia obliterante degli arti inferiori (AOAI), diagnosticata mediante indice caviglia-braccio (ABI), livelli sierici di Vitamina D e marcatori di rimodellamento osseo (teclopate C-terminale del collagene di tipo I-sCTX ed isoenzima osseo della fosfatasi alcalina-hALP).

**Materiali e Metodi.** Sono stati inclusi 99 pazienti di età media di 75±8.8 anni, di entrambi i sessi, afferenti agli ambulatori o al reparto di degenza della Medicina Interna, Angiologia, Malattie da Arteriosclerosi, dell’Università degli Studi di Perugia.

**Risultati.** I valori medi di 25(OH)Vitamina D sono risultati ridotti nella popolazione in studio (15.5±9.3 ng/mL; vs 30-100 ng/mL), mentre aumentati sono risultati i valori medi di sCTx in donne in post-menopausa (0.8±0.5 mg/mL; vs 0.32±0.15) e negli uomini (0.33±0.19 ng/mL; vs 0.22±0.15). I pazienti con ABI patologico (≤0.90) presentavano valori significativamente più bassi di 25(OH)Vitamina D (12.9±10.1 ng/mL vs 17.6±8.7 ng/mL, p=0.023) e significativamente più elevati di sCTX (0.9±0.5 ng/mL vs 0.7±0.4 ng/mL, p=0.05) rispetto ai pazienti con ABI normale (≥0.90). Si evidenziava una correlazione positiva, statisticamente significativa, tra i livelli di vitamina D e l’ABI (Rho=0.547, p=0.001) ed una correlazione negativa, statisticamente significativa, tra i livelli di sCTX e l’ABI (Rho=0.238, p=0.026). Tali correlazioni persistevano anche dopo aggiustamento per i fattori di rischio classici dell’arteriosclerosi. I risultati preliminari del nostro studio confermano l’elevata prevalenza dell’ipovitaminosi D nella popolazione anziana, associata ad un incremento del turnover osseo.

**Conclusioni.** I livelli sierici di CTX sembrano predire la presenza dell’AOAI e potrebbero rappresentare uno dei possibili meccanismi patogenetici responsabili della frequente e simultanea presenza di osteoporosi e arteriosclerosi in tale popolazione.

**ULTRASONOGRAPHY MODIFICATIONS OF VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE AFTER PIOGLITAZONE OR GLIBENCLAMIDE THERAPY COMBINED WITH ROSUVASTATIN IN TYPE 2 DIABETIC PATIENTS NOT WELL CONTROLLED BY METFORMIN**

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**Aim.** To evaluate the effects of pioglitazone or glibenclamide alone and in combination with rosuvastatin on hepatic steatosis in type 2 diabetic patients.
Resources were evaluated at the end of the study period.

Methods. One hundred and sixty patient were enrolled. After a run-in 3 months period of metformin 850 mg three times a day, patients were randomized to take pioglitazone 15 mg twice a day or glibenclamide 5 mg twice a day for six months, then rosvastatin was added for other six months. Patients underwent an ultrasound examination (steatosis degree, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) diameter), an euglycemic hyperinsulinenic clamp (glucose infusion rate (GIR)), and a blood sample (glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), post-prandial glucose (PPG), fasting plasma insulin (FPI), lipid profile) at baseline, and after 6, and 12 months, respectively.

Results. Both pioglitazone and glibenclamide induced a significant and similar reduction of HbA1c, FPG, and PPG compared to baseline (p<0.05 for all). Pioglitazone reduced FPI (p<0.05), while glibenclamide increased it (p<0.05). Pioglitazone significantly increased GIR compared to glibenclamide therapy. The addition of rosvastatin did not significantly worsened the glycemic control. Regarding lipid profile, pioglitazone significantly reduced total cholesterol (TC), and triglycerides (TG), and increased high density lipoprotein cholesterol (HDL-C), while glibenclamide did not affect it. When rosvastatin was added, an improvement of lipid profile was observed in both groups, but the improvement was greater with pioglitazone and rosvastatin (p<0.05 vs glibenclamide plus rosvastatin). Pioglitazone significantly decreased the steatosis degree, SAT, and VAT diameter compared to baseline (p<0.05 for all), while glibenclamide did not. The addition of rosvastatin to pioglitazone further decreased the steatosis degree, SAT, and VAT diameter compared to baseline (p<0.01 for all) and gave also a decrease of these parameters in the glibenclamide group (p<0.05 for all).

Conclusions. Pioglitazone was more effective than glibenclamide in improving the hepatic steatosis indices.

OLMESARTAN AND ATORVASTATIN ARE EFFECTIVE IN IMPROVING CIRCULATING PROGENITOR CELLS PROFILE IN HYPERTENSIVE SUBJECTS

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Background. MicroRNAs (miRs) 221 and 222 have been identified in circulating progenitor cells (CPCs); miRs are involved in the regulation of cell proliferation, differentiation and angiogenesis. Previously we found that in CPCs from hypertensive patients without additional risk for CAD, miR221/222 are increased and associated with cell number and production of reactive oxygen species (ROS). The aim of the present study was to evaluate whether in hypertensives a treatment with olmesartan may have effects on the number of CPCs and on levels of miR221/222 and ROS. We also evaluated whether additional effects may be obtained with an add-on treatment with atorvastatin.

Methods. We included 41 hypertensives with no additional risk factor for CAD and 22 matched controls; we evaluated circulating CD34+ cell number, intracellular miR221/222 and ROS levels at baseline (T0) and after a six months treatment with 20 mg/die of olmesartan (T1); blood pressure, fibrinogen, CRP, glucose and lipid profile were also evaluated. Then, hypertensives were randomized to receive an add-on treatment with atorvastatin 20 mg/die (T2a), or to continue with olmesartan alone (T2p) for further 3 months. All parameters were evaluated at the end of the study period.

Results. At T1, systolic and diastolic blood pressure, ROS and miR221/222 were significantly decreased (all p<0.001) with respect to T0, while the number of cells was increased (p<0.001). CRP and fibrinogen levels were also reduced (p<0.001). After the treatment with atorvastatin (T2a) ROS, miRs, CRP and fibrinogen levels were further decreased (all p<0.005), and CPCs significantly higher (p=0.007); blood pressure values also were further reduced, while lipid profile amelioration didn’t reached the statistical significance. At T2p no further changes were detected as compared to T1.

Conclusions. Olmesartan is effective in reducing miRs and ROS levels in CPCs from hypertensives, as well as in increasing CPC number. An add-on treatment with atorvastatin may improve these effects.

INFLUENCE OF HEPATIC STEATOSIS ON PRE-CLINICAL ATHEROSCLEROSIS

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Non-alcoholic fatty liver disease is associated with metabolic abnormalities configuring the framework of metabolic syndrome, leading to accelerated atherosclerosis and increasing cardiovascular risk. Whether the fatty liver may exert an additive effect, independent of other metabolic risk factors, in determining the vascular damage, is discussed.

We evaluated in 145 dyslipidemic patients the degree of liver steatosis by ultrasonography. In addition to anthropometric and metabolic parameters and ultrasonographic assessment of visceral fat area (VFA), we measured aortic pulse wave velocity (PWV), and brachial flow-mediated dilation (FMD) as measures of arterial stiffness and endothelial dysfunction, and carotid intima-media thickness (IMT). On the basis of the values of these three parameters we realized a score, named Atherosclerosis Score (AS), whose value is indicative of a greater vascular damage.

Subjects with a higher degree of steatosis had increased levels of plasma triglycerides, glucose, systolic blood pressure, decreased levels HDL cholesterol levels and higher VFA. The prevalence of metabolic syndrome increased with increasing degree of steatosis, reaching 77.8% in patients with moderate to severe steatosis. Age (standB=0.328, p<0.001) and the area of visceral fat (standB=0.428, p<0.001) were independent predictors of the degree of steatosis. Worsening liver steatosis is associated with decreasing flow-mediated dilation (p<0.001) and increasing arterial pulse wave velocity (p<0.001) and IMT (p<0.001). Atherosclerosis Score is significant correlated with the degree of steatosis, VFA, BMI, systolic and diastolic blood pressure, blood glucose and the presence of metabolic syndrome. Multivariate analysis showed that the degree of steatosis (standB=0.257, p=0.018), age (standB=0.390, p<0.001), and systolic blood pressure (standB=0.284, p=0.023) were independent predictors of the AS.

We can conclude that in dyslipidemic patients, hepatic steatosis is associated with pre-clinical atherosclerosis independent of confounding metabolic risk factors. The degree of hepatic steatosis, determined by ultrasound, is in turn influenced by age and the area of the visceral fat.
IDENTIFICATION OF A NEW POINT MUTATION IN THE LCAT GENE AND DIAGNOSIS OF FISH-EYE DISEASE DUE TO COMPOUND HETEROZYGOSITY

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Introduction. Fish-eye disease (FED) is an autosomal recessive disorder characterized by corneal opacity and markedly decreased plasma concentrations of high-density lipoprotein cholesterol (HDL-C), apolipoprotein (apo) AI, and apo AII. The biochemical features of FED are due to a selective inability of the enzyme lecithin:cholesterol acyltransferase (LCAT; EC 2.3.1.43) to esterify cholesterol in HDL, resulting in pronounced decreases of HDL-C levels in homozygous and compound heterozygous patients. Despite severe HDL-C deficiency, affected subjects do not show an increased risk of atherosclerotic cardiovascular disease.

Patients. Here we report the case of two Italian sisters, CC and CB, aged 50 and 55 years, respectively, presenting with corneal opacities, extremely low HDL-C levels (<5 mg/dl) and apo-AI plasma concentrations of 40-45 mg/dl. Genetic analysis of the LCAT gene was performed in both women to help diagnose the cause of their dyslipidemia.

Methods. Genomic DNA was isolated from peripheral white blood cells using the MagnaPure LC DNA Extractor. All six exons of the LCAT gene including all intron-exon boundaries were PCR-amplified from 150-200 ng of genomic DNA on a GeneAmp PCR System 9700. Amplification products were purified and then sequenced on both strands using the 3730 DNA Analyzer.

Results. Sequencing of the LCAT gene in the two probands revealed compound heterozygosity for a missense mutation in exon 6 (c.997G>A; V332M) previously reported to underlie the FED phenotype, and a novel point mutation located at the splice donor site of intron 2 (g.1882T>C). Based on genetic and clinical findings, both women were diagnosed with FED. Genetic analysis on the LCAT gene was extended to a third sister, their mother and 3 children. All these family members were found to be heterozygous carriers of the V332M mutation only and showed low-normal plasma HDL-C levels (35-45 mg/dl).

PRIMARY HYPERLIPEIDEMIAS IN CHILDREN: EFFECT OF PLANT STEROL SUPPLEMENTATION ON PLASMA LIPOIDS AND MARKERS OF CHOLESTEROL SYNTHESIS AND ABSORPTION

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Introduction. The relationship between hyperlipidemia and cardiovascular events is unquestioned, and it is now clear that dietary and eventually drug treatment needs to be started as soon as possible. Lowering of low-density lipoprotein (LDL) cholesterol is the primary therapeutic goal of lipid-lowering therapy in patients at risk for cardiovascular disease. Phytosterols are plant components presenting a chemical structure similar to that of cholesterol, and they are known to lower serum cholesterol levels by competing with cholesterol for intestinal absorption. Their lipid-lowering efficacy has been demonstrated in adults but only a few data are available in children. Aim of this study was to evaluate the efficacy, tolerability and safety of plant sterol supplementation in children with different forms of primary hyperlipidemia.

Methods. The effect of plant sterol consumption on plasma lipid levels was evaluated in 32 children with heterozygous FH, 13 children with Familial Combined Hyperlipidemia (FCH) and 13 children with Undefined Hypercholesterolemia (UH) in a 12-week open-label intervention study using plant sterol-enriched yoghurt. Plasma lipids and apolipoproteins were measured by routine laboratory methods. Markers of cholesterol synthesis (lathosterol) and absorption (campesterol and sitosterol) in plasma were measured by GC-MS with multiple selected ion monitoring.

Results. Tolerability and adherence to recommended regimen was very high. A significant reduction in LDL-cholesterol levels was observed in the three groups (10.7, 14.2 and 16.0% in FH, FCH and UH, respectively). Lathosterol concentrations were unchanged, reflecting a lack of increased synthesis of cholesterol. Of the two absorption markers analysed, only sitosterol showed a slight but significant increase. Daily consumption of plant sterol dairy products favourably changes lipid profile by reducing LDL-cholesterol. To our knowledge, this is the first report of the use of plant sterols-enriched food in treating children with primary hyperlipidemias such as FCH and UH.

A NEW POSSIBLE ROLE FOR ANGIOPOIETIN LIKE 3, A REGULATOR OF TRIGLYCERIDE METABOLISM, IN MODULATING INSULIN RESISTANCE

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In experimental models angiopoietin like 3 (Angptl3) has been shown to directly enhance the release of free fatty acids (FFA) and glycerol from adipose cells. As increased FFA are involved in the pathogenesis of insulin resistance, a reduced plasma concentration Angptl3 could affect insulin-sensitivity. We have recently identified a cohort of subjects carrying the S17X loss of function (LOF) mutation in the Angptl3 gene. Therefore, we have taken this opportunity to test the hypothesis of an involvement of Angptl3 deficiency in modulating insulin sensitivity.

We enrolled 207 non-carriers, 59 heterozygous and 6 homozygous carriers of the S17X mutation. Fastin blood samples were obtained for measuring plasma glucose, insulin, lipids, Angptl3, hsCRP and FFA. As index of insulin sensitivity, we calculated HOMA-IR as reported. Comparisons were adjusted for age, gender and BMI using generalized linear model.

As expected, both homozygotes and heterozygotes showed a significant reduction in plasma lipoprotein levels as compared to non-carriers. Homozygous carriers had no circulating Angptl3, while heterozygotes had 42% reduction in Angptl3 level compared to non-carriers (p<0.0001). Homozygous, but not heterozygous carriers showed a significant reduction of FFA (1.4 ± 0.6 mmol/L vs 0.6 ± 0.4 mmol/L; P=0.007).

No difference in fasting plasma glucose was observed among groups. Conversely, as compared to heterozygotes and non-
carriers, homozygotes showed significantly lower plasma insulin (homozygotes 3.2 (0.5-10.2) μU/ml; heterozygotes 5.3 (1.6-23.9) μU/ml; non-carriers 3.9 (0.2-55.3) μU/ml; adjusted p=0.013) and HOMA-IR (0.86(0.05÷2.20) in homozygotes; 1.32 (0.38÷6.49) in heterozygotes and 1.36 (0.04÷9.45); adjusted p= 0.005). We found no differences in hsCRP levels.

In summary, these results indicate the complete deficiency of Angptl3 is associated with improved insulin sensitivity suggesting that Angptl3 may play a role also in modulating insulin sensitivity.

References

**EFFECT OF ROSUVASTATIN ON MICRONURAS EXPRESSION IN HUMAN ATHEROSCLEROTIC PLAQUES: THE QUASAR STUDY**

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Background. Statins are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and are a leading therapy for the prevention of ischemic events. Although lowering of plasma low-density lipoproteins (LDL) is the most relevant effect, numerous studies suggest many other pleiotropic effects of statins, which involve improving of muscle mitochondrial function, enhancing atherosclerotic plaque stability and decreasing oxidative stress and inflammation. MicroRNAs are small non-coding RNAs which act as post-transcriptional gene regulators. Growing evidences support the microRNAs involvement in cardiovascular disease and there are tantalizing hints of the effect of statin on microRNAs. The aim of this study is to investigate whether a short-time treatment with low or high dose of rosuvastatin may affect microRNAs expression in human atherosclerotic plaques.

Material and Methods. In the “QUalitative Analysis of plaque Stability After Rosuvastatin therapy in asymptomatic patients enlisted to undergo carotid endarterectomy” (QUASAR) 70 patients with severe stenosis of the internal carotid artery were randomized to receive a 12 week low (10 mg/day) or high (40 mg/day) doses of rosuvastatin before the elective endarterectomy. Total RNA was extracted from plaques using Trizol reagent. Pools creation for MiRNome qPCR analysis was carried out using total RNA extracted from respectively 11 selected plaques of rosuvastatin 10 mg and 40 mg samples and from 11 plaques of naive hypercholesterolemic patients (control group). MiRNome qPCR analysis was performed by using miRCURY LNA Universal RT microRNA PCR system. MicroRNAs validation study was performed on all plaques from the pooled samples by qPCR.

Results. MiRNome qPCR analysis of 742 microRNAs on pooled hypercholesterolemic samples versus respectively pooled rosuvastatin 10mg and rosuvastatin 40mg samples showed several microRNAs dysregulated in rosuvastatin groups versus hypercholesterolemic one. We have paid attention on nine microRNAs on the strength of their predicted target genes involved in atherosclerosis. Real-time PCR validation studies on all plaques from the pooled samples showed that both rosuvastatin doses significantly up-regulated mir-3 (p=0.004), mir-20b (p<0.001), mir-133a/b (p=0.001), mir-144 (p<0.001), mir-30a (p=0.01) and mir-377 (p=0.002) with respect to hypercholesterolemic patients. Mir-150 and mir-155 were not found significantly down-regulated in the qPCR validation analysis.

Conclusions. These data showed that short-term rosuvastatin treatment may affect microRNAs expression profile in human atherosclerotic plaques. Further studies on their predicted target gene are required to better understand microRNAs involvement in the beneficial effects observed with statin-based lipid lowering therapies.
I dati sull'obesità riportano che il 31,6% della popolazione risultala in sovrappeso, mentre il 10,6% è obeso. L'eccesso ponderale, la frequenza dello SNP2 non presenta variazioni significative nei due gruppi (rs4149056: 23/23/24% e 22/22/23%). I valori mediani di CPK nei due gruppi relativamente agli alleli dello SNP1 evidenziano differenze significative nei CT: “casi” 322 UI/L (197-606) vs “controlli” 93 UI/L (41-142) p=0,000; nei wild type: “casi” CPK 304 UI/L (211-515) vs “controlli” 71 UI/L (49-96) p=0,000. Dei 32 “casi” wild type per lo SNP1 28 risultavano però essere mutati per lo SNP2 (12 CC e 16 CT).

I nostri dati sembrano confermare l'influenza esercitata da questi due polimorfismi sulla miopatia da statine.

C-REACTIVE PROTEIN AND INSULIN RESISTANCE, RATHER THAN METABOLIC SYNDROME, PREDICT BOTH TOTAL AND CARDIOVASCULAR MORTALITY IN THE ELDERLY. DATA FROM 9-YEARS FOLLOW-UP IN THE INCHIANTI STUDY

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Aim. Although in middle-age population metabolic syndrome (MetS) is a strong risk factor for overall and cardiovascular (CV) mortality, in the elderly population this association is at least doubtful. Concurrently MetS is physiologically linked to both insulin resistance (IR) and systemic inflammation (SysInf). Thus we analysed the relationship between MetS, IR, and SysInf in 9 years CV and total mortality in 1011 community dwelling elderly subjects (age: 75.5±7.4 year; female: 56%).

Methods. IR was defined as HOMA grater then median value of the population (2.27). SysInf was defined as hs.CRP plasma levels>3mg/L. 9-years CV and total mortality was evaluated with multivariate Cox models (age, sex, cholesterol, BMI, smoking and diabetes adjusted).

Results. 311 individuals presented MetS by NCEP-ATPIII criteria. 10% of population had MetS without IR, while 28% had IR without MetS. Among subjects IR+, MetS was associated with higher HOMA values; among subjects IR-, MetS wasn’t associated with SysInf. Interestingly, MetS+/IR- individuals had higher levels of hs.CRP compared with MetS+/IR+ subjects. The adjusted risk for MetS, based on presence/absence of IR and SysInf, was: - IR-/SysInf+: (MS:17%): OR:1(reference). - IR+/SysInf+ (MS:24%): OR:1.67 (1.07-2.61). - IR+/SysInf- (MS:35%): OR:2.74 (1.83-4.10). - IR-/SysInf+ (MS:49%): OR:5.09 (3.39-7.63).

Results after 9-years follow-up: MetS wasn’t associated with CV and total mortality whereas combination of IR-/SysInf+ significantly predicts both total and CV mortality in both MetS+ and MetS- subjects.

Conclusions - Older subjects with MetS might be included into two categories: a) subjects IR+ (64%) with high values of HOMA and hs.CRP, and b) subjects IR- (32%) with high levels of hs.CRP (32%).

I POLIMORFISMI DEL GENE SLC01B1 INFLUENZANO LA TOLLERABILITÀ ALLA TERAPIA CON STATINE

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I polimorfismi a singolo nucleotide (SNPs) del gene SLC01B1 che codifica per Organic Anion-Trasporting Polipeptide 1B (proteina regolante la captazione epatica delle statine) sembrano essere mutati per lo SNP2 (12 CC e 16 CT).
- IR and Sysnfi have a combined effect on the prevalence/risk of MetS among older individuals.
- IR and Sysnfi predict total and CV mortality independently of MetS. Thus determining hs.CRP and HOMA in the elderly could become useful in clinical practice.

**OXIDATIVE STRESS MARKERS AND ENDOPLASMIC RETICULUM STRESS MANAGEMENT IN CORONARY ATHEROSCLEROSIS**

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**Purpose.** Oxidative stress, altered redox state and a weakened anti-oxidant defence are implicated in atherogenesis. Nowadays the role of Unfolded Protein Response (UPR) and Endoplasmic Reticulum (ER)-initiated apoptosis in the pathophysiology of cardiovascular disease is under investigation. This study was aimed to evaluate plasma inflammatory, oxidative stress and redox state markers (high sensitivity C Reactive Protein, hsCRP; oxidized phospholipids, oxPAPC and glutathione, GSH) of Chronic Coronary Artery Disease patients (CAD). Moreover UPR and ER-initiated apoptosis and the expression of the protective system Nrf2/ARE were explored in Peripheral Blood Mononuclear Cells (PBMC) of the same subjects.

**Methods.** 29 CAD and 23 controls were enrolled. Oxidative stress and cellular redox state were evaluated by measuring hsCRP, oxPAPC and GSH plasma levels. UPR and ER-initiated apoptosis were evaluated by BiP and CHOP expression in PBMC and so also the protective factor Nrf2 and HMOX-1 expression.

**Results.** hsCRP and oxPAPC concentrations resulted significantly higher in CAD than controls. CAD had lower GSH than controls. The expression of CHOP and BiP were significantly higher in CAD than in controls, with inadequate Nrf2/ARE response.

**Conclusions.** The higher levels of hsCRP and oxPAPC and the lower concentrations of GSH in CAD compared to controls indicated that inflammation, oxidative stress and altered redox state were maintained in chronic coronary artery disease but with an inadequate protective response in terms of Nrf2 expression. UPR and ER-initiated apoptosis were stimulated in CAD, suggesting that this response remains activated in chronic stress situations.

**EVALUATION OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS (EPCS), CIRCULATING PROGENITOR CELLS (CPCS) AND CIRCULATING ENDOTHELIAL CELLS (CECS) IN AMD PATIENTS**

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**Background.** To evaluate circulating endothelial and progenitor cells as biomarkers in exudative and atrophic AMD patients, in order to establish their possible clinical usefulness.

**Material and Methods.** Blood samples from 12 patients with dry AMD and 19 newly diagnosed neovascular AMD were analyzed before as well as 16 weeks, after three intravitreal injections of ranibizumab. At baseline and after treatment the patients underwent standard ophthalmological examination, fluorescein angiography and OCT scan; the wet AMD patients were distinguished in two groups (responders and non responders) according to visual acuity and OCT foveal thickness modifications. The number of circulating endothelial progenitor cells (EPCS: CD34+/KDR+; CD133+/KDR+; CD34+/KDR+/CD133+), circulating progenitor cells (CPCS: CD34+; CD133+; CD34+/CD133+) and the circulating endothelial cells (CECs) were determined by flow cytometry at baseline in all the patients and after treatment in the wet AMD group.

**Results.** No statistically significant difference for EPCS and CECs was found between patients with dry and wet AMD; whereas CPCs were higher in exudative AMD vs atrophic. AMD patients, especially those of the cellular subset CD133+ (p=0.0035). A significant EPCs reduction was observed after treatment (p 0.011 for CD34+/KDR+, p 0.012 for CD133+/KDR+, p 0.038 for CD34+/KDR+/CD133+). Finally no significant difference was found between responders and non responders to the antiangiogenic treatment, even if the group with the worst clinical outcome showed a higher number of CPCs at baseline.

**Conclusions.** We have reported a higher number of CPCs in exudative than dry AMD and a significant reduction of EPCS after three intravitreal injections of ranibizumab. Circulating endothelial and progenitor cells may represent an useful biomarker of choroidal neovascularization and of its response to antiangiogenic treatment.

**HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: PLASMA EXCHANGE IN CASE OF DRUG FAILURE**

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**Introduction.** Familial hypercholesterolemia (FH) is an autosomal codominant disorder characterized by lifelong elevation of LDL-cholesterol concentrations, tendon xanthomas, and early-onset atherogenic disease.

**Case report.** A 59 years old women with a history of familiar hypercholesterolemia, non smoker, presented to outpatient for the persistence of elevated cholesterol despite statin treatment in the last seven years. Blood examination showed total-Cholesterol 377 mg/dl, HDL-Cholesterol 59 mg/dl, Triglycerides 96 mg/dl, LDL-Cholesterol 290 mg/dl, in therapy with atorvastatin 20 mg (treatment by general practitioner). At physical examination we observed right tendon xanthoma and xanthelasmas. Genetic analysis for LDL-receptor mutation found an heterozygous mutation IVS 15-3 C>A with residual LDLR activity of 52%. Ecg exercise test revealed ST segment depression in DII, DIII, AVF, V4 and V6. The patient underwent percutaneous coronary angiography and drug-eluting stents were placed on right coronary and anterior descending arteries. We changed drug treatment to a combination of rosuvastatin plus ezetimibe. Despite this, therapeutic target was
not attained, so combined plasma exchange therapy with HMG-CoA reductase inhibitor was started. At present the patient is free from cardiovascular events since five years.

**Discussion.** FH is characterized by high risk of premature coronary artery disease (CAD) and LDL-apheresis or Plasma exchange (an effective surrogate) increases life-expectancy in homozygous. Some studies showed that LDL-apheresis could inhibit the progression of cardiovascular disease also in heterozygous FH refractory to drug therapy. LDL-apheresis or Plasma exchange have been approved by The Food and Drug Administration for homozygous FH patients with LDL>503 mg/dl, heterozygous FH with LDL>302 mg/dl and heterozygous FH with cardiovascular disease and LDL>201 mg/dl, despite intensive drug therapy.

**Conclusion.** Plasma exchange is a useful tool in heterozygous FH patients with high LDL-C levels, despite intensive drug therapy, or in patients with drug intolerance to prevent CAD and to reduce atherosclerosis progression.

**Dietary Supplementation with Tetradecylthioacetic Acid or Salmon Peptides Reduces Atherosclerosis Progression in ApoE-Ko Mice**

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**Aim.** Tetradecylthioacetic acid (TTA) is a synthetic long-chain fatty acid analogue with marked hypolipidemic and anti-inflammatory effects. These combined effects are of particular interest in view of a potential anti-atherogenic activity. Fish peptides have shown to have hypolipidemic effects and thus may slow the progression of atherosclerosis.

**Aim of the study was to investigate the effect of TTA and of salmon peptides on atherosclerosis progression in apoE-Ko mice.**

**Methods.** 36 apoE-Ko mice have been randomly divided in three groups and fed for 12 weeks a high-fat diet alone (control) or supplemented with TTA 0.3 % and salmon protein hydrolysate 5% (100 g of diet). At sacrifice, aortic arch and heart have been harvested and processed to perform en-face and histological analysis, respectively.

**Results.** Compared to the control group, salmon peptide hydrolysate supplementation led to a significantly reduced plaque development at both the aortic arch (+66.6% vs. control; p<0.05) and the aortic sinus (+36.9% vs. control; p<0.05). The supplementation with TTA 0.3% led to a significantly reduced atherosclerotic plaque area in the aortic arch (+77.7% vs. control; p<0.05) and displayed a trend towards a lower plaque development in the aortic sinus (+25.9% vs. control; p=0.068).

**Conclusions.** The present study strongly suggests that a supplementation with TTA 0.3% reduces atherosclerotic plaque development in high-fat fed mice, particularly at the aortic arch. Supplementation with 5% salmon peptide hydrolysate significantly reduced atherosclerotic lesion progression at both the aortic sinus and the aortic arch.

**Expansion of Necrotic Core and Shedding of Extracellular Domain of MERTK Receptor in Human Carotid Plaques: A Role for Oxidative Derivatives of Polyunsaturated Fatty Acids?**


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**Aim.** Expansion of necrotic core (NC), a major feature responsible for plaque disruption, is likely the consequence of accelerated macrophages apoptosis coupled with defective phagocytic clearance (efferocytosis). The cleavage of the extracellular domain of Mertk by metalloproteinase domain17 (Adam17) has been shown to produce a soluble Mertk protein (sMer), which can inhibit efferocytosis. Herein we analyzed the expression and localization of Mertk and Adam17 in the tissue around the necrotic core (TANC) and in periphery (P) of human carotid plaques. Then we studied the mechanisms of NC expansion by evaluating which components of TANC induce Adam 17 and the related cleavage of the extracellular domain of Mertk.

**Methods and Results.** We studied 97 human carotid plaques. The expression of Mertk and Adam17 resulted higher in TANC than in P (p<0.01). By immunohistochemistry, Mertk resulted higher than Adam17 in the area of TANC near to lumen (p<0.01) but much lower in the area close to NC (p<0.01). The extract of this portion of TANC increased the expression (mRNA) of Adam17 and Mertk (p<0.01) in macrophage-like THP-1 cells but it also induced the cleavage of the extracellular domain of Mertk generating sMer in the medium (p<0.01). This effect of TANC extract was most evoked by its content in F2-isoprostanes, hydroxyoctadecadienoic acids and hydroxytetraenoic acids.

**Conclusions.** Some oxidized derivatives of polyunsaturated fatty acids contained in TANC of human carotid plaques are strong inducers of Adam17, which in turn leads to the generation of sMer, which can inhibit efferocytosis.

**Plasma Homocysteine Distribution and Its Association with Parental History of Cardiovascular Disease in a Population of Children with Eterozigous Familial Hypercholesterolemia**

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**Aim.** increased plasma homocysteine levels are considered an independent risk factor for cardiovascular disease (CVD) in adults. Aim of this study is to evaluate plasma homocysteine levels and correlation with family history of CVD in children with heterozygous familial hypercholesterolemia (FH) at their first access to our Lipid Clinic.

**Methods.** 119 FH children (genetical diagnosis), 2-17 years of age (median 8), 55 males and 64 females, were evaluated for: family
history for CVD (according to AAP guidelines 2008), anthropometric measures, pubertal stage (Tanner +1 vs +1), plasma lipid profile by enzymatic method, plasma total homocysteine (tHcy) levels, folic acid and vitamin B12 levels. None was receiving pharmacological treatment or vitamin supplementation. Statistics: Student’s t test for independent samples.

**Results.** Family history was positive in 65 (CVD+) (first degree relatives in 19, second in 46) and negative in 54 (CVD-) children. Blood lipid profile was not different by age, sex, pubertal stage or family history for CVD. Plasma tHcy levels (mean±SD, mcM/L) was 5.96±2.1, with no difference in CVD+ and CVD- children. tHcy levels were significantly higher in pubertal (Tanner +1, 32 children) than in pre-pubertal stage (Tanner +1, 87 children), respectively: 7.07±2.5 vs 5.55±1.6, p<0.001. Serum folate and vitamin B12 levels were within normal range.

**Conclusions.** In our population there seem to be no correlation between tHcy levels and family history for CVD. tHcy levels seem to relate with pubertal activation. Molecular analysis for MTHFR could be useful in pediatric population to detect both homozygous and heterozygous subjects, who could benefit from vitamin supplementation to prevent CVD in adulthood.

### STATIN THERAPY VERSUS CHOLESTYRAMINE THERAPY IN A POPULATION OF ITALIAN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA: EFFECT ON BLOOD LIPOID PROFILE

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**Aim.** According to International Guidelines, pharmacological intervention should be considered for children aged 10 or more who, despite 6-12 months of dietary treatment, present elevated LDL-C levels (LDL-C>190 mg/dl without other risk factors, LDL-C>160 with other risk factors). The widely used medications are bile acid sequestrants and statins, that sholud result in cholesterol lowering respectively 10%-20% and 20%-50% below baseline. The aim of this study is to compare the effect of pravastatin vs cholestyramine therapy on lipid profile in children with familial hypercholesterolemia (FH) followed in our Lipid Clinic.

**Methods.** Twenty FH children (10 M, 17 F, age 10-15, median age 12.6 y), already on low-fat diet for 12 months, were followed-up for three months after pharmacological treatment, 14 with pravastatin 20 mg/die (group 1), 13 with cholestyramine 4g/die (group 2). Blood samples for lipid profile were collected at baseline and after three months. The lipid profile before pharmacological therapy was (mean values ± standard deviation, mg/dl): TC 299.4±56, LDL-C 221.9±54, HDL-C 58.3±9.1, Tryglicerides 97.8±8.9. Statistics: Student’s t test for independent samples.

**Results.** After three months of treatment LDL-C was significantly reduced in both groups (p<0.001). Mean (mg/dl)± sd of TC, LDL-C, HDL-C and tryglicerides before and after treatment were in group 1: 299.6±43 vs 227.3±41, p<0.001; 225.8±48 vs 175.4±54, p<0.001; 55.4±8 vs 53.5±8, p=0.98; 101.1±45 vs 73.3±57.6, p<0.05; in group 2: 299.1±70 vs 251±56, p<0.001, 217.7±61 vs 170.6±53 p<0.001; 61.6±9 vs 62.6±13 p=0.72, 94.3±72 vs 82.5±58 p=0.32 in group 2. The percentual variation in TC and LDL-C in group 1 and 2 was, respectively: -20.8% vs -15.1% p=0.141, -20.7% vs-20.8% p=0.98, with no difference between the two treatments.

**Conclusions.** We found that both pravastatin and cholestyramine reduce TC and selectively LDL-C, with no effect on HDL-C. Unexpectedly, these preliminary findings suggest that there are no significant differences on LDL-C after treatment in our small sample. This positive effect is worth for further investigations.

### NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH AN INCREASED PREVALENCE OF ATRIAL FIBRILLATION IN PATIENTS WITH TYPE 2 DIABETES

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**Background/Aims.** Although non-alcoholic fatty liver disease (NAFLD) and atrial fibrillation (AF) share multiple cardiometabolic risk factors such as insulin resistance, obesity, hyperlipidemia, diastolic dysfunction, and arterial stiffness, the role of NAFLD in AF remains unclear. We hypothesized that children and adolescents with type 2 diabetes (T2D) and NAFLD have a higher prevalence of AF compared to those with T2D and no NAFLD.

**ABSTRACT**

**PZIENTI AFFETTI DA IPERLIPEMIA FAMILIARE COMBINATA E CARATTERIZZATI DA BASSE HDL PRESENTANO UN NUMERO RIDOTTO DI UNITÀ FORMANTI COLONIE ENDOTELIALI ISOLABILI EX-VIVO

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**Background.** The number of Unità Formanti Colone (UFC) isolated ex vivo from the sample peripherico represents a useful tool to evaluate the function of the precursors endothelial circulanti. Some misurazione correla inoltre con i fattori di rischio cardiovascolare. L’iperlipemia Familiare Combinata (FCH) è un disordine del metabolismo lipidico che si associa ad un aumentato rischio cardiovascolare. Scopo del nostro lavoro è stato quello di valutare il numero di eCFU nei pazienti affetti da FCH.

**Metodi.** Dodici pazienti con FCH e basso colesterolo HDL, e dodici pazienti controllo con normale colesterolo HDL sono stati arruolati in questo studio. In questi pazienti è stato valutato il numero di eCFU isolabili e la loro correlazione con i livelli plasmatici di colesterolo HDL, ApoA1, colesterolo totale, colesterolo LDL, trigliceridi, ApoB100 e con la dilatazione flusso-mediata (FMD) o nitroglicerina-mediata (NMD).

**Risultati.** Nei pazienti FCH il numero di eCFU è risultato ridotto rispetto ai pazienti controllo (16.29±7.6 vs 28.7±14, P<0.01). La dilatazione della arteria brachiale valutata come rapporto FMD/NMD è risultata significativamente ridotta nei pazienti FCH rispetto ai controlli (0.83 ± 0.03 vs 1.01±0.07, P<0.01). All’analisi univariata, il numero di eCFU è risultata correlare con i livelli plasmatici di: colesterolo HDL (r=0.231, P=0.03), apoA1 (r: 0.241, P<0.029), trigliceridi (r:0.271, P=0.014), colesterolemia totale (r:0.27 P=0.014), colesterolo LDL (r:0.228, P<0.04), rapporto colesterolo totale/colesterolo HDL (r:0.314, P<0.029), apoB100 (r: 0.335, P<0.002) e con il rapporto FMD/NMD (0.257, P=0.032).

**Conclusioni.** Il numero ridotto di eCFU osservato nei pazienti FCH indica una disfunzione dei progenitori endoteliali circulanti di questi pazienti. Tale disfunzione potrebbe essere in parte responsabile dell’alterazione vascolare osservata in questa condizione clinica.
bolic risk factors, however, it is currently unknown if there is an association between NAFLD and increased prevalence of AF in people with type 2 diabetes mellitus (T2DM), a group in which NAFLD and AF are very common.

**Methods.** We identified all patients with T2DM discharged from our Division of Endocrinology during 2007-2011 after excluding: 1) patients with any clinical evidence of malignancies, end-stage renal disease, acute hepatitis of any etiology, cirrhosis or other secondary causes of chronic liver disease (n=126), and 2) those for whom a liver ultrasound examination was unavailable (n=25). As a result, 702 hospitalized patients with T2DM met our study criteria and were included in statistical analysis. NAFLD was defined by ultrasonographic detection of hepatic steatosis in the absence of other liver diseases. The diagnosis of AF was confirmed in affected patients by experienced cardiologists.

**Results.** Of the 702 patients included in the study, 514 (73.2%) had NAFLD and 85 (12.1%) had AF. NAFLD was associated with an increased risk of prevalent AF (odds ratio (OR) 3.04, 95% CI 1.5-6.0, p=0.0001). Adjustments for age, sex, hypertension (defined as BP ≥140/90 mmHg or treatment), electrocardiographic left ventricular hypertrophy, obesity, chronic kidney disease, chronic obstructive pulmonary disease, use of lipid-lowering drugs, pre-existing history of congestive heart failure, ischemic heart disease, valvular heart disease and hyperthyroidism did not attenuate the association between NAFLD and AF (adjusted OR 4.39, 95%CI 2.1-9.4, p=0.0001).

**Conclusions.** This study is the first to demonstrate a strong association between NAFLD and increased prevalence of AF in patients with T2DM independent of several clinical risk factors for AF. Future studies are needed to corroborate these results, and to determine whether NAFLD predicts the development and persistence of AF in people with T2DM.

**CRYOAblaTioN INDUCES A TRANSIENT ACTivation of THE HAE mootic SYSTeM**

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**Background.** Cryoablation (CA) is a promising technique for the treatment of supraventricular arrhythmia. Until now scarce data on the effect of cryoblation on the hemostatic system are available. The aim of this study was to investigate hemostatic system alterations in patients who undergo CA.

**Materials and Methods.** Prothrombin fragment F1+2 (F1+2), Thrombin-Antithrombin complex (TAT), D-Dimer and plasminogen activator inhibitor type-1(PAI-1) plasma levels were determined in 16 atrioventricular nodal reentrant tachycardia patients (7M/9F, mean age 57+/-20 years) who underwent CA. Blood samples were obtained before the procedure (T0), at the end of electrophysiological study (T1), immediately after the procedure of cryoablation (T2) and 24 hours afterwards (T3).

**Results.** During follow-up (68+/-38 days) no recurrences were observed. After cryoablation (T2), F1+2, TAT and D-Dimer plasma levels significantly increased with respect to T0 (T2: F1+2: 644 (177-799) pmol/l, TAT: 45.1 (9.0-112.9) µg/mL, D-Dimer: 436(126-3125) ng/mL vs T0: F1+2: 155 (88-701) pmol/l, TAT: 4.6 (1.4-101.0) µg/mL, D-Dimer: 121(39-1689) ng/mL (p < 0.001)) and with respect to T1 (F1+2: 535 (176-735) pmol/l, TAT: 30.0 (4.0-117.2) µg/mL, D-Dimer: 234 (21-3186) ng/mL, p<0.05) in all patients. F1+2, TAT and D-Dimer levels were significantly (p<0.001) higher at T1 with respect to T0. After 24 hours from the cryoablation (T3) F1+2, TAT and D-Dimer levels significantly decreased (F1+2: 180(78-508) pmol/l, TAT: 3.4(1.1-14.8) µg/mL, D-Dimer: 200 (49-726) ng/mL, p<0.01) and reach the pre-procedure levels (T0).

**Conclusions.** Our data indicated that CA determined a transient hypercoagulable state with alterations on F1+2, TAT and D-Dimer in patients who underwent CA. These alterations were not associated with changes in PAI-1 plasma levels suggesting a negligible influence of the CA on the fibrinolytic system and, likely, on the endothelial activation.

**IPOLIpIDEMIA FAmIlIARe CoMBINATA DOVUTA A Nuove muTAZIoNI DEL GENE ANGPTL3**

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**Introduzione.** L’ipobetalipoproteinemia Familiare (FHBL) è un disordine co-dominante caratterizzato da bassi livelli di colesterolo LDL (LDL-C) e apolipoproteine A1 (ApoA1). Nel 50% dei casi la FHBL è dovuta a mutazioni del gene APOB; in una piccola parte dei casi essa è dovuta a mutazioni con perdita di funzione del gene PCSK9. Recentemente, il gene ANGPTL3 è emerso come nuovo gene candidato nella FHBL quando questa è associata a bassi livelli di HDL-colesterolo (HDL-C); tale condizione è nota come ipolipidemia Familiare Combina.

**Scopo.** Analisi del gene ANGPTL3 in un soggetto di origine greca che presentava una ipolipidemia combinata.

**Metodi e Risultati.** Il probando ZB presentava un’ipobetalipoproteinaemia moderata (TC 131 mg/dl, LDL-C 95 mg/dl, ApoB 62 mg/dl) associata a bassi livelli di HDL-C (24 mg/dl). L’analisi dei geni APOB e PCSK9 e PCSK9 è risultata negativa. Il probando è invece risultato portatore eterozigote di due nuove mutazioni del gene ANGPTL3 (c.1147 A>T e c.1198 G>A), entrambe localizzate nell’eson 6, che determinano due sostituzioni aminoacidiche non conservative (p.Thr383Ser e p.Gly400Arg). Tali mutazioni sono verosimilmente patogene, poiché coinvolgono aminoacidi altamente conservati nello sviluppo e che sono presenti sia in silico che in vivo.

**Conclusioni.** 1) le mutazioni di ANGPTL3 individuate nel probando sono state poste in posizioni diverse, quindi egli è un portatore e 2) lo screening di tutte le mutazioni nei familiari del probando apporta un contributo importante nel profilo lipidico dei portatori.
ON-TREATMENT C-REACTIVE PROTEIN AND HDL CHOLESTEROL LEVELS: ASSOCIATION WITH CAROTID INTIMA-MEDIA THICKNESS

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Aims. Statin therapy is followed by reductions in carotid intima-media thickness (CIMT) and C-reactive protein (CRP) levels, but a significant number of treated patients still have increased CIMT. We investigated whether on-treatment levels of CRP predict CIMT in hypercholesterolemic patients receiving statin therapy. The influence of blood pressure and anti-hypertensive therapy on the association between CRP and CIMT was evaluated.

Methods. Assessment of cardiovascular risk factors, CRP and CIMT was performed in a cross-sectional study of 230 hypercholesterolemic patients at intermediate cardiovascular risk under statin therapy; 125 patients received only a statin (Statin Group) and 115 also anti-hypertensive therapy (Combined Therapy Group).

Results. Logarithmically transformed CRP (beta=0.17, p=0.01) and HDL cholesterol levels (beta=-0.27, p=0.001) were predictors of CIMT, irrespective of confounders. High CRP levels (>3 mg/L) were associated with a 2.7-fold increased risk of having high CIMT (>1.25 mm). High CIMT was present in a high percentage of patients not at target for cholesterol and blood pressure levels (61%). Patients in the Statin Group had lower Framingham risk and CIMT than those in the Combined Therapy Group. In the Statin Group, logarithmically transformed CRP (beta=0.28, p=0.004) and HDL cholesterol (beta=-0.21, p=0.03) were associated with CIMT. In the Combined Therapy Group, HDL cholesterol was the only significant CIMT predictor (beta=-0.33, p=0.001).

Conclusions. On-treatment CRP and HDL cholesterol levels are associated with CIMT among hypercholesterolemic patients under statin therapy. In patients receiving both statin and anti-hypertensive therapy, HDL cholesterol remains the main covariate of CIMT.

A RARE CASE OF SEVERE HYPERCHILOMICERONEMIA AND ACUTE PANCREATITIS ASSOCIATED TO A NOVEL MUTATION OF LMF1 GENE

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The proband was a 41 year old male from Romania hospitalized in our department for severe acute pancreatitis (Balthazar D) with onset with intense epigastralgia and retrosternal irradiation. Eruptive xanomas were visible on back and to the roots of limbs, hepatic steatosis was revealed at ultrasound examination. The patient is suffering from second-degree obesity (BMI 36.8 kg/m²) and during hospitalization was found decompensated diabetes mellitus (HbA1c 11.5%), severe hypertriglyceridemia (TG 71.9 mmol/L) and high total cholesterol (24.3 mmol/L). Examination of the fundus oculi had not shown lipemia retinalis or diabetic retinopathy. The patient underwent therapeutic plasmapheresis and started the pharmacological treatment with insulin glargine 16 U/day, metformin 1500 mg/day, atorvastatin 40 mg/day with reduction of triglyceride levels in 24 hours (5.37 mmol/L). One month after discharge, the lipid profile was Total Cholesterol 8.0, HDL-C 0.77, TG 13.3 mmol/L. No data of family members were collected at this time. Despite the presence of diabetes and potus, in view of the severity of clinical features, we performed the sequencing analysis of candidate genes. LPL, APOA5, APOC2, GPIHBP1 genes were negative for pathogenic mutations, while the sequencing of LMF1 revealed a new mutation in homozgyous status: c.121_122delCG p.Pro41Arg fs*39. This mutation is probable disease-causing because generates a truncated protein. Functional studies are not available at this time but may be performed.

A CASE REPORT OF SEVERE HYPERCHILOMICERONEMIA WITH RECURRENT PANCREATITIS: PROBABLE AUTOIMMUNE PATHOGENESIS DEMONSTRATED BY REMISSION WITH IMMUNOSUPPRESSIVE THERAPY

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The aim is to describe a case of marked hypertriglyceridemia and recurrent acute pancreatitis in a young girl from Albania. The severe hypertriglyceridemia (97.70 mmol/L) associated to pancreatitis occurred the first time at 20th year of age, was treated with plasma exchange procedure and a combination of hypolipidemic treatments (fenofibrate and Omega 3 fatty acids), including strict hypolipidemic dietary therapy. At discharge from the first episode, triglyceridemia was 1.65 mmol/L but after only three weeks serum triglycerides increased up to 50.06 mmol/L and caused a second pancreatitis. Genomic DNA was sequenced for LPL, GPIHB1, APOA5, APOC2, and LMF1 genes but no disease-causing variants were identified. All lipid-lowering treatments were inefficacious and the patient underwent to several apheretic treatments without good control of triglycerides levels (between 17.1 and 39.9 mmol/L) because she was intolerant to procedures. A few cases of autoimmune type I hyperlipidemia occurring in patients with autoimmune disease have been reported. Since a clinical history of autoimmunity was present in our patient (ANA, anti-TPO, anti-SSA antibodies positive) we assumed an autoimmune pathogenesis of hypertriglyceridemia and we introduced immunosuppressive therapy (azathioprine 100 mg/day and corticosteroid 50 mg/day). After two weeks, triglyceride levels decreased to 1.6 mmol/L and remained below 0.9 mmol/L during treatment with immunosuppressants, although we have progressively reduced dosage of corticosteroids. After two months of complete remission, the patient voluntarily discontinued azathioprine but after six weeks there was a relapse of the disease (triglycerides levels 66.12 mmol/L, abdominal pain and increase of pancreatic enzymes). Immunosuppressive therapy was re-started. Laboratory analysis in order to identify anti-LPL autoantibodies before and during immunosuppressive therapy are ongoing.
**MITRAL VALVE DISEASE: FOCUS ON OXIDATIVE STRESS, PLATELET ACTIVATION AND NITRIC OXIDE PATHWAY**

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**Introduction.** Although clinical features and pathophysiology of degenerative mitral valve insufficiency are known, limited data are available regarding biochemical perturbations implicated in the progression of this disease. A putative role was suggested for oxidative stress, haemostasis and nitric oxide (NO) pathway perturbations. The aim of this study was to evaluate the analytes involved in the above cited conditions and to highlight their possible relationships in mitral valve disease.

**Material and Methods.** In this observational study 45 patients affected by mitral valve regurgitation due to prolapse (MVR) were compared with 65 healthy subjects with cardiovascular risk factors similar to patients (Ctrol). Oxidative stress (ratio of reduced and oxidized glutathione: GSH/GSSG; isoprostane: 8-iso-PGF2α; platelet activation (11-dehydro thromboxane B2: 11-DH TXB2), and nitric oxide synthesis (arginine, ADMA, SDMA, ornithine, citrulline and tetrahydrobiopterin) were evaluated by HPLC or LC-MS/MS methods. ANOVA general linear models and Spearman’s correlations were used for statistical analysis.

**Results.** MVR patients had higher levels of oxidative stress than Ctrl subjects as documented by lower GSH/GSSG ratio (p<0.0001) and increased 8-iso-PGF2α levels (p<0.0001). 11-DH-TXB2 was also elevated in MVR group (p=0.02). We found a significant positive correlation between 8-iso-PGF2α and 11-DH-TXB2 in both groups (p<0.05). MVR patients showed elevated levels of NO synthesis inhibitor ADMA (p=0.006), lower citrulline (p=0.03) and higher ornithine concentration (p=0.02) than Ctrols; a significant positive correlation was found between GSH/GSSG and Arginine/ADMA ratios (p<0.05).

**Conclusions.** MVR patients have increased oxidative stress that could induce an enhanced platelet activation and a perturbation in the synthesis of NO, which, in turn, worsens the already impaired haemostasis in these patients. In conclusion, MVR is a multi-fac-torial process and a more complete knowledge of the involved molecular pathways may allow the discovery of targeted therapeutic strategies aimed at modifying or slackening MVR natural course in the early phases.

**EFFECT OF SPHINGOSINE 1-PHOSPHATE (S1P) RECEPTOR AGONISTS FTY720 AND CYM5442 ON ATHEROSCLEROSIS DEVELOPMENT IN LDL RECEPTOR DEFICIENT (LDL-R −/−) MICE**

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**Objectives.** Sphingosine 1-phosphate (S1P) – a lysosphingolipid present in HDL – exerts atheroprotective effects in vitro, while FTY720, a non-selective S1P mimetic inhibits atherosclerosis in LDL receptor-deficient (LDL-R −/−) mice under conditions of severe hypercholesterolemia. We here examined the effect of FTY720 and a selective S1P receptor type 1 agonist CYM5442 on atherosclerosis in moderately hypercholesterolemic LDLR −/− mice.

**Methods and Results.** LDLR −/− mice fed Western diet (0.25% cholesterol) were given FTY720 (0.4 mg/kg/day) or CYM5442 (2.0 mg/kg/day) for 18 weeks. FTY720 but not CYM5442 persistently lowered blood lymphocytes, depleted CD4+ and CD8+ T cells in spleen and lymph nodes, and reduced splenocyte IL-2 secretion. However, both compounds reduced the activity of splenic and peritoneal macrophages as inferred from the down-regulated CD68 and MHC-II expression in CD11b+ cells and the reduced IL-6 secretion in response to LPS, respectively. CYM5442 and FTY720 reduced weight gain, white adipose tissue depots and fasting glucose suggesting improvement of metabolic control, but failed to influence atherosclerosis in LDL-R −/− mice.

**Conclusion.** Despite down-regulating macrophage function and - in case of FTY720 - altering lymphocyte distribution CYM5442 and FTY720 fail to affect atherosclerosis in moderately hypercholesterolemic LDL-R −/− mice. We hypothesize that S1P mimetics exert atheroprotective effects only under conditions of increased cholesterol burden exacerbating vascular inflammation.

**ROLE OF FIBRONECTIN-EDA IN NEO-INTIMA HYPERPLASIA - LESSONS FROM ANIMAL MODELS**

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**Background.** Fibronectins (FN) are multifunctional glycoprotein present in the plasma and in the ECM of the tissues. The primary transcript undergoes alternative splicing to generate iso forms namely FN-EA, FN-EDB, III-ICS. FN-EDA is regulated spatially and temporally during development and ageing. FN generally exist in two major forms namely Plasma FN and cellular FN. Plasma FN is soluble and lacks both EDA and EDB while cellular FN possess these fragments.
FN-EDA levels are increased during a series of path physiological conditions while FN-EBD levels are mainly increased during development. The molecular mechanisms mediated by FN-EDA are yet to be known. We investigate the role of FN-EDA in restenosis.

**Materials and Methods.** Specific gene targeting allowed the generation of mice that express fibronectin constitutively containing EDA, or lacking EDA in the plasma and in the peripheral tissues. In order to investigate the role in neointima formation a non-obstructive collar was placed on the right carotid artery of FN-EDA+/−, FN-EDA−/− and FN-EDAwt/wt mice for a period of 9 weeks. Formalin-fixed paraffin embedded carotid and sham-operated sections were stained with haematoxylin and eosin staining and morphometrically evaluated by OPTIMAS 6.2 image software. Data were analyzed for ANOVA followed by tukey’s multiple comparison tests. The vascular smooth muscle cells isolated from skin biopsies, were cultured according to the standard methods.

**Primary TDho and TDhe fibroblast cell lines, obtained of ABCA1 mutation (TDho) and his heterozygous father (TDhe).**

Skin fibroblasts senescence in vitro in an Italian homozygous case of sclerotic CAD has been proposed, so we investigated replicative relationship between cellular senescence and development of atherosclerosis at the onset of ATS development is prevented by brief discontinuations of atherogenic diet regimen able to modulate plasma lipids and systemic inflammation. Apparently, the discontinuity in the administration of the high-fat diet resulted in a better adaptive response.

**RESULTS.** Compared to TDhe fibroblasts, TDho cells showed phenotype associated to senescence, cell proliferation and increased staining for SA-beta-gal. The cells percentage SA-beta-gal positive was highly increased in TDho compared to TDhe cells (66.15% vs 41.53% respectively). ABCG1 gene expression in TDho fibroblasts where higher than in TDhe cells, at first replication cycles (0.44 vs 0.14 arbitrary unit respectively), but showed a down-regulation at late cycles (0.14 vs 0.08 arbitrary unit respectively). We observed an up-regulation of LDLr fibroblasts expression at late replicative cycles respect to first replication cycles, but a small differences between TDho and TDhe (38.4 vs 34.3 arbitrary unit and 15.4 vs 13.6 arbitrary unit respectively).

**Conclusions.** TDho fibroblasts showed accelerated senescence in vitro in a gene dosage way. These data highlight the need for further studies on relation between altered HDL metabolism, senescence and CAD in TD.
LDL APHERESIS RESTORES THE BALANCE IN IMMUNE RESPONSE, ENDOThelial ACTIVATION AND OXIDATIVE STATUS

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Aim. LDL-apheresis (LDL-A) has beneficial effects on cardiovascular events prognosis and on endothelial function by mechanisms that still to be completely understood.

Methods. We studied 8 patients (males, mean age 56±12 years) affected by Familial Hypercholesterolemia (FH), before and serially up to the rebasing baseline conditions after LDL-A. Lipid profile, myeloperoxidase (MPO) -index of leukocytes activation and endothelial adhesion molecules levels (sICAM1 and sELAM1) were monitored. From protein extracts of isolated mononuclear cells, eNOS, phosphorylated eNOS (p-eNOS), HO-1 -major cytoprotective and antioxidative pathways-, AKT and phosphorylated AKT (pAKT) were determined. Intracellular anti-inflammatory cytokines (IL-4 and IL-10) and pro-inflammatory cytokines (INF-gamma and IL-6) were assayed.

Results. LDL-A mean percent reductions of TC (total cholesterol), LDL-C, HDL-C, TG (triglycerides), and Lipoprotein (a) were 74%, 82%, 7%, 86%, and 86%, respectively. Two days after LDL-A treatment, MPO, sICAM1 and sELAM1 were significantly reduced (p<0.001) and showed parallel cholesterol behavioural pattern rebound. Mononuclear subpopulation cells varied from a 10% CD4 increase to a 12% CD8 decrease. TC was inversely correlated with pAKT (50-70%) were increased. From protein extracts of isolated mononuclear cells, eNOS, phosphorylated eNOS (p-eNOS), HO-1 -major cytoprotective and antioxidative pathways-, AKT and phosphorylated AKT (pAKT) were determined. Intracellular anti-inflammatory cytokines (IL-4 and IL-10) and pro-inflammatory cytokines (INF-gamma and IL-6) were assayed.

Conclusions. Cholesterol removal obtained with LDL-A induced an increase of cytoprotective and antioxidant signals, as also in anti-inflammatory cytokines levels, at the same time MPO and adhesion molecules concentrations were reduced. Is therefore plausible that LDL-A treatment contributes to the restoration of endothelium anti-inflammatory status.

A RARE EVENT IN CLINICAL LIPIDOLOGY: FOUR SIBLINGS WITH HOMOZYGOUS AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA

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Introduction. Autosomal Dominant Hypercholesterolemia (ADH) is a monogenic disorder with a frequency of 1:300/500 in heterozygous form. ADH homozygotes are exceedingly rare. Mutations in the LDLR gene are the most frequent cause of ADH (ADH-1).

Methods. LDLR gene was resequenced in an ADH family with 4 siblings presenting with a lipid profile suggesting severe heterozygous ADH.

Results. The four siblings had elevated plasma TC (from 335 to 552 mg/dl) and LDL-C (from 276 to 460 mg/dl). TC and LDL-C in their mother were 220 and 154 mg/dl respectively. The siblings were found to be compound heterozygotes for two LDLR mutations:
1) a novel 24 nt deletion (c.1175_1186del1186+1_12del) which eliminates the last 12 nt of exon 8 and the first 12 nt of intron 8;
2) a point mutation (c.1003 G>A, p.G335S) known as Paris-6. The 24 nt deletion was assumed to affect mRNA splicing as it eliminated the donor splice site in intron 8.

SANA ALIMENTAZIONE IN GRAVIDANZA E RELAZIONI CON IL NEO NATO

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Lo studio è stato condotto tra Aprile 2011 e Febbraio 2012 presso il reparto di Ginecologia e Ostetricia ospedaliera dell’OO.RR. Foggia, Italy, al fine di valutare gli effetti positivi della sana alimentazione nella gravidanza e le correlazioni con la salute del nascituro. Sono state reclutate 100 donne sane di razza caucasica in prima gestazione nella gravida e le correlazioni con la salute del nascituro. Sono state reclutate 100 donne sane di razza caucasica in prima giornata di ricovero, che avessero espletato il parto (spontaneo e cesareo) tra la 38-41ª settimana. Criteri di esclusione: patologie a carico dell’apparato cardio-circolatorio, polmonare, patologie metaboliche, proprie della gravidanza, infezive, parto pretermine, parto in analgesia, malformazioni neonatali. Verificatesi le condizioni per il reclutamento, sono state informate sugli obiettivi dello studio; veniva calcolato il BMI, riportato peso pre-gestazionale, invitate a compilare un questionario di frequenza alimentare, sottoposte ad anamnesi nutrizionale e recall delle 24 ore. Dall’analisi degli obiettivi dello studio; veniva calcolato il BMI, riportato peso pre-gestazionale, invitate a compilare un questionario di frequenza alimentare, sottoposte ad anamnesi nutrizionale e recall delle 24 ore. Dall’analisi gli obiettivi dello studio; veniva calcolato il BMI, riportato peso pre-gestazionale, invitate a compilare un questionario di frequenza alimentare, sottoposte ad anamnesi nutrizionale e recall delle 24 ore. Dall’analisi di correlazione tra i due gruppi, troviamo significatività per il gruppo con BMI <25 e il peso dei neonati che si aggirano attorno 3700±330 g a differenza del gruppo di donne con BMI ≥25,99 in cui il peso dei neonati è 3430±269 g. (p<0,05). (BMI <24,99 = 3,43±0,26; BMI ≥25,99 = 3,70±0,33)*. Dall’analisi di correlazione tra i gruppi sussidiarvisi per BMI e i valori di APGAR medi, troviamo significatività statistica nelle donne con BMI <24,99 e gli APGAR sono superiori alle donne con BMI ≥25,99. (p<0,05). (BMI <24,99 = 3,43±0,26; BMI ≥25,99 = 3,70±0,33). La corretta alimentazione in gravidanza e conseguente buono stato nutrizionale è garante di alto valore APGAR.
ADIPOCYTE FATTY ACID-BINDING PROTEIN 4 AND EPIPLIEMIA FAMILIARE COMBINATA

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The adipocyte fatty acid-binding protein 4 (FABP4) is a new adipochina responsible for the transport intracellular of acids grassi ed è altamente espresa nei macrofagi e negli adipociti. In studi animali è stato dimostrato che essa gioca un ruolo importante sia nel metabolismo lipidico che in quello glucidico. Anche nell'uomo, recentemente è stato osservato che l'FABP4 viene rilasciata in circolo e che livelli circolanti elevati di questa proteina sono associati con l'obesità centrale, l'insulinoresistenza e l'aterosclerosi.

In letteratura, vi è un solo studio nel quale sono stati studiati i livelli sierici di FABP4 in pazienti affetti da iperlipidemia familiare combinata (FCHL).

La nostra ricerca ha lo scopo di confrontare i livelli plasmatici di FABP4 e di alcune variabili metaboliche connesse al rischio cardiovascolare tra i pazienti FCHL e un gruppo di controllo. Sono stati esclusi dallo studio i soggetti affetti da iperlipidemia secondaria, quelli trattati per dislipidemia e gli obesi (BMI>30). Inoltre, gli FCHL presentano rispetto al gruppo controllo valori sostanzialmente più alti di FABP4 negli FCHL rispetto ai controlli (19.9 ng/mL±10.1 vs 10.7 ng/mL±4.9, p<0.02).

Questi dati, ancora preliminari, suggeriscono che elevati livelli sierici di FABP4 nei pazienti trattati per dislipidemia potrebbero rappresentare un potenziale marker di squilibrio metabolico e contribuire, al pari del profilo lipoproteico, al rischio cardiovascolare di tali soggetti.

PERFORMANCE EVALUATION OF AUTOMATED IMMUNOASSAYS FOR THE DETECTION OF ANTI PF4/HEPARIN COMPLEX ANTIBODIES

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They are now available automated immunoassays for the determination of antibodies anti-PF4/Hep.

This study was aimed to evaluate the performance of two automated immunoassays in detecting anti-PF4/Hep antibodies. In 96 patients investigated for suspected HIT we performed: 1-ELISA assays (total and IgG) (GTI Diagnostic), 2-rapid automated latex enhanced immunoassay (Instrumentation Laboratory), 3-chemiluminescence tests HemosIL AcuStar-Ab/AcuStar-IgG and AcuStar HIT-IgG/AcuStar-IgG and 4-functional assay heparin-induced platelet aggregation (HIPA).

51/96 (53.1%) patients had a positive test with ELISA total method, 40/96 (41.7%) with ELISA IgG (≥0.4 OD.) and 32/96 (33.3%) with latex immunoassay (≥21.0 U/ml). 29/96 (30.3%) and 23/96 (24.0%) patients had a positive test with AcuStar-Ab and AcuStar-IgG respectively. In 13 (13.5%) patients the HIPA test was positive. A HIPA positive test was detected in 13/51 (25.5%) patients with ELISA (total) positive assay, in 13/40 (32.5%) with ELISA IgG positive assay, in 13/32 (40.6%) patients with positive latex immunoassay, in 13/29 (44.8%) patients with positive AcuStar-Ab and in 13/23 (56.5%) patients with positive AcuStar-IgG.

The peak of thrombin generation (Cmax, mA/min) significantly increased from baseline to T1 (p=0.05) remaining unchanged throughout the ovarian stimulation protocol. ETP (mA) significantly increased from baseline to T1 (p<0.001), thus reducing at T2 and T3, even if values remained higher than baseline. Tag and Tmax did not exhibit significant alterations.

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The peak of thrombin generation (Cmax, mA/min) significantly increased from baseline to T1 (p=0.05) remaining unchanged throughout the ovarian stimulation cycle (p<0.001). The peak amount of thrombin generation (Cmax, mA/min) significantly increased from baseline to T1 (p<0.001), thus reducing at T2 and T3, even if values remained higher than baseline. Tag and Tmax did not exhibit significant alterations. TFPI values progressively and significantly decreased throughout the ovarian stimulation cycle (p<0.003). No significant correlation between ET and TFPI was observed. CLT significantly lengthened from baseline to T1.
La chirurgia bariatrica, o chirurgia “metabolica”, sta assumendo un ruolo rilevante nel trattamento dell’obesità di grado severo in grado di ridurre peso corporeo e comorbidity correlate. Recentemente è stato introdotto l’intervento di sleeve gastrectomy (SG) caratterizzato dalla riduzione del volume gastrico del 75-80%. Poco note sono a tutt’oggi le modifiche dell’assetto nutrizionale a distanza dalla SG.

Scopo del nostro studio è stato quello di valutare le eventuali modificazioni dei parametri nutrizionali, antropometrici e metabolici di 17 soggetti con obesità di grado severo (BMI 46.6 kg/m², 41.0-49.7) tutti sottoposti ad intervista strutturata “Dietary History” prima e dopo circa dieci mesi dalla SG.

Nel post-intervento si è evidenziata riduzione statisticamente significativa del BMI (BMI 34.4 kg/m², p=0.001), della circonferenza vita (p=0.001), di glicemia (p=0.004) insulinemia (p=0.005) e HOMA-index (p=0.005). La valutazione dei parametri lipidici ha evidenziato incremento del colesterolo HDL (p=0.047) mentre non si sono documentate modificazioni significative del C-LDL e trigliceridi. Non modificazioni significative del livello di acido folico e vitB12.

Dall’indagine alimentare è emersa una riduzione significativa del 49.7% tutti sottoposti ad intervista strutturata “Dietary History” prima e dopo circa dieci mesi dalla SG.

I pazienti, per stabilire strategie terapeutiche adeguate alle esigenze di ogni singolo paziente, sono sottoposti a SG, peraltro preliminari e che necessitano di un ampliamento nella numerosità, rivestono fondamentale importanza non solo affinché la perdita di peso sia quanto più possibile sicura ma anche per stabilire strategie terapeutiche adeguate alle esigenze di ogni singolo paziente.

**Assessment Nutrizionale Prima e Dopo “Sleeve Gastrectomy” in Pazienti con Obesità di Grado Severo**

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Scopo del nostro studio è stato quello di valutare le eventuali modificazioni dei parametri nutrizionali, antropometrici e metabolici di 17 soggetti con obesità di grado severo (BMI 46.6 kg/m², 41.0-49.7) tutti sottoposti ad intervista strutturata “Dietary History” prima e dopo circa dieci mesi dalla SG.

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Aim. To evaluate the biochemical determinants of aspirin-resistance in a population of type 2 diabetic men on aspirin therapy. Methods. In 81 type 2 diabetic men (age: 63.5±0.7 years; diabetes duration: 12.5±2.1 years; BMI: 29.1±0.4 kg/m²) on chronic aspirin (100 mg/die), defined aspirin-resistant if they presented PFA-100 CEPI closure time <200 seconds, we determined:
1. metabolic parameters: HbA1c, fasting blood glucose, total, HDL and LDL cholesterol, triglycerides and apo B-100;
2. oxidative stress markers: urinary isoprostanes (8-epi-PGF2alpha) and plasma Superoxide Dismutase activity (SOD);
3. markers of platelet activation: sCD40L, sP-Selectin, serum and urine Thromboxane (TX) B2.
Results. 60 men (74%) were ASA-sensitive and 21 men (26%) ASA-resistant. ASA-resistant vs ASA sensitive patients differed for:
1. total cholesterol (181.0±6.6 vs 159.0±4.1 mg/dl, p=0.007), LDL-cholesterol (117.0±6.5 vs 93.2±3.9 mg/dl, p=0.003), non-HDL-cholesterol (142.8±7.1 vs 117.7±3.7 mg/dl, p=0.01), APO-B100 (93.0±4.9 vs 77.2±3.0 mg/dl, p=0.009);
2. plasma SOD activity (0.21±0.01 vs 0.24±0.01 U/ml, p=0.05), urinary 8-epi-PGF2alpha isoprostane (1.35±0.11 vs 0.84±0.09 ng/mg creatinine, p=0.003);
3. serum TXB2 (5242±2934 vs 1550±313, p=0.01) and 11-DHTX B2 (2144.8±306.0 vs 1387.2±66.11 pg/mg creatinine, p=0.0001).
PFA-100 CEPI significantly correlated (p=0.050.0001) with non-HDL-cholesterol, APO-B100, serum and urinary TXB2, and 8-epi-PGF2alpha. Both HbA1c and non HDL cholesterol significantly contributed to the oxidative stress (measured as isoprostane values) in a linear multivariate analysis.
Conclusions. In type 2 diabetes mellitus determinants of aspirin resistance are parameters of lipid control (non HDL-cholesterol and Apo-B100) and oxidative stress markers. Both HbA1c and non HDL-cholesterol contribute to explain the increased oxidative stress observed in this condition.

MICRO-RNA 143/145 DEFICIENCY IS ASSOCIATED WITH REDUCED ATHEROSCLEROSIS IN LDL-RECEPTOR KNOCK-OUT ANIMALS

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MicroRNAs (miRNAs) have emerged as a novel class of endogenous, small, non-coding RNAs that negatively regulate gene expression via degradation or translational inhibition of their target mRNAs. MicroRNA-143/145 have an important role in the modulation of vascular smooth muscle cell (VSMC) phenotype whose deregulation is associated with vascular disorders such as atherosclerosis. In order to study the influence of these miRNAs on atherosclerosis development we have generated miR143/145 knock out (KO) mice on atherogenic background (LDL receptor KO). The animals were fed with an high-fat diet for 16 weeks and then blood lipids content and atherosclerosis development were investigated.Cholesterol and triglycerides blood levels were similar among the animal groups. The morphometric analysis at tricuspid valve level showed a 40% decrease in atherosclerotic lesion of double KO animals compared with LDL-R KO. Further characterization of the lesion showed a similar collagen content and necrotic core extension. Gene expression analysis of the atherosclerotic lesions in the arterial wall is on going to identify the genes and the molecular mechanisms associated with these findings.

In summary, miRNA 143-145 deficiency is associated with an atheroprotective effect in LDL-receptor knock-out animals.

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INVESTIGATION OF VASCULAR CHANGES ASSOCIATED WITH FAMILIAL COMBINED HYPOLIPIDEMIA

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Familial combined hyperlipidemia (FHBL2, OMIM #605019), due to mutations in ANGPTL3 gene, is a recently described low-cholesterol syndrome characterized by a reduction of both pro-atherogenic (VLDL and LDL) and anti-atherogenic (HDL) lipoproteins. The impact of FHBL2 on the risk of atherosclerosis is not well defined. We assessed in 66 FHBL2 subjects carrying the ANGPTL3 S17X LOF mutation (7 homozygotes and 59 heterozygotes) and 126 age- and sex-matched controls the extent of preclinical atherosclerosis by measuring carotid intima-media thickness (IMT) and flow-mediated dilatation (FMD). Three IMT values on each side were obtained and average (Avg-IMT) and maximum (Max-IMT) were calculated. FMD was measured according to the guidelines of the International Brachial Artery Reactivity Task Force. Plasma concentration of soluble forms of VCAM-1, ICAM-1, and E-selectin were also measured as biomarkers of endothelial function. Finally, the capacity of serum obtained from 15 FHBL2 individuals (7 homozygotes, 8 heterozygotes) and 8 non-carriers to promote cell cholesterol efflux was tested. Compared with controls, homozygous FHBL2 carriers showed increased Avg-IMT (0.81±0.20 vs. 0.59±0.33 mm, p=0.01) and max-IMT (1.57±0.41 vs. 0.82±0.66 mm, p=0.001). Homozygous carriers also showed lower, although not significant, FMD. As a group, FHBL2 showed significantly higher concentration of sE-selectin when compared to non-carriers. In addition, homozygous FHBL2 presented the highest concentration of sE-selectin and sVCAM, even though only the former reached the statistical significance; no difference were observed in sICAM-1 levels. Compared to controls, ApoB-depleted sera from FHBL2 individual had a reduced capacity to promote cell cholesterol efflux (4.9±1.8% vs. 8.5±1.6%; p=0.01). This was evident with ABCA1-, SR-BI- and ABCG1-mediated pathways and was related to the number of mutated alleles.

In summary, homozygous FHBL2 appear to have more advanced preclinical atherosclerosis and this might be related to their impaired HDL function.
HIGH DOSE ROSUVASTATIN MODULATES MICRORN-222 EXPRESSION IN ADVANCED HUMAN ATHEROSCLEROTIC PLAQUES

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ABSTRACT

Background. MicroRNAs (miR) are small non-coding RNAs which act as post-transcriptional gene regulators. Evidence supports a role for miR-222 in modulation of vascular biology. In particular, it has been demonstrated that miR-222 is able to reduce inflammatory-induced intraplaque vascularization, a marker of vulnerable atherosclerotic plaque, and to promote smooth muscle cells proliferation. Both experimental and clinical evidence clearly shows that rosuvastatin is able to reduce atherosclerosis burden and to improve plaque stability. Despite many of the long-term effects of statin are due to its cholesterol-lowering abilities, some beneficial effects appear not to be directly linked to mere cholesterol reduction and are not fully elucidated. Thus, in this study we have evaluated the effect of low and high-dose rosuvastatin on expression of miR-222 in human atherosclerotic plaques.

Material and Methods. In the “QUalitative Analysis of plaque Stability After Rosuvastatin therapy in asymptomatic patients enrolled to undergo carotid endarterectomy” (QUASAR) 70 patients with severe stenosis of the internal carotid artery were randomized to receive a 12 week low (10 mg/day) or high (40 mg/day) doses of rosuvastatin before the elective endarterectomy. In this analysis we have evaluated 11 patients treated with high-dose rosuvastatin and 11 matched hypercholesterolemic controls. Total RNA was extracted from plaques using Trizol and miR-222 expression was evaluated by RealTime-PCR.

Results. Real-time PCR study has demonstrated that miR-222 is significantly upregulated in atherosclerotic plaques from patients treated with rosuvastatin (p<0.02). Post-hoc test confirmed that miR-222 expression levels are significantly increased in patients treated with high-dose of rosuvastatin when compared with control group.

Conclusions. To best of our knowledge, here we provide the first evidence that high-dose rosuvastatin increases the expression of miR-222 in human atherosclerotic plaques. Due to its effects on intraplaque neovascularization, miR-222-lowering effect may represent a further beneficial effect of high-dose rosuvastatin in plaque stabilization.

INDICI DI RIGIDITÀ E RISCHIO CARDIOVASCOLARE GLOBALE: RISULTATI DA UNA POPOLAZIONE ABRUZZESE IN PREVENZIONE PRIMARIA

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Background. La rigidità arteriosa rappresenta un promettente marcatore di danno d’organo nel paziente con fattori di rischio cardiovascolare convenzionali ed emergenti e la sua valutazione è raccomandata dalle più recenti linee guida sull’iper tensione arteriosa. Nell’ambito di questo studio abbiamo valutato la corre lezione tra gli indici di rigidità arteriosa e il rischio cardiovascolare globale (RCG) stimato allo scopo di identificare un parametro che possa meglio definire il profilo di rischio cardiovascolare.

Metodi. Mediante l’utilizzo del sistema Vicorder (Medical Graphics Italia) sono stati valutati gli indici di stiffness arteriosa (Pulse Wave Velocity Carotid-Femoral, PWV, e Augmentation Index, AI) di 221 soggetti consecutivi in prevenzione primaria (età media 54,0±13,9 anni; maschi 357%, ipertesi 63,8%) giunti presso il nostro centro nel periodo marzo-giugno 2012 per una valutazione cardiovascolare. Per ogni soggetto sono stati raccolti i principali parametri antropometrici, la pressione arteriosa in cibo e postprandio e il RCG a 10 anni mediante il Framingham Risk Score (FRS) e il calcolatore individuale del rischio del progetto Cuore (CUORE). I dati sono stati analizzati mediante software SPSS 16.0 per Windows.

Risultati. La PWV media è risultata 8,47±3,86 m/s, l’AI medio era 25,1±7,7%. I valori di PWV correlavano positivamente con il RCG stimato mediante FRS (r=0,345 p<0,001) e CUORE (r=0,298 p<0,001) così come i valori di AI (r=0,288 p<0,001) e r=0,321 p<0,001 rispettivamente. Considerando i soli pazienti ipertesi (n=142, età media 58,7±13,5, maschi 54,9%), la correlazione tra PWV, AI e RCG si rafforza considerando sia il FRS (r=0,389 p<0,001; r=0,219 p<0,001 rispettivamente), sia l’algoritmo CUORE (r=0,477 p<0,001; r=0,374 p<0,001). Tra i due algoritmi (FRS e CUORE) è stato riscontrato un elevato grado di correlazione (r=0,882 p<0,001). Come atteso, i valori di PWV correlavano positivamente con l’età (r=0,336 p<0,001) e con la presenza di ipertensione arteriosa.

Conclusioni. La stiffness arteriosa, valutata mediante tecnica non invasiva, correla positivamente con il RCG stimato a 10 anni nei pazienti in prevenzione primaria. Tale osservazione rafforza la potenzialità della valutazione della stiffness arteriosa nella stratificazione prognostica del paziente a rischio cardiovascolare e, soprattutto, del paziente iperteso.

LDL AHERESIS IN LIPOPROTEIN GLOMERULOPATHY: A CASE REPORT

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Introduction. Lipoprotein glomerulopathy (LG), described by Saio, is characterized by lipoprotein in glomerular capillaries, type III hyperlipoproteinemia and high apolipoprotein E (apo E) serum
levels. LG is a rare disease, mainly observed in Asian population; it presents with nephrotic syndrome naturally evolving towards end stage of renal failure.

**Methods.** A 44 years Caucasian male was referred to our outpatient Clinic for Inherited Lipid Disorders in Pisa with LG diagnosis for evaluation of LDL apheresis appropriateness. The patient arrived to our observation on therapy with ACE inhibitors, sartanic and hypolipemic drugs (simvastatin/ezetimibe) while his renal function was progressively worsening (creatinine 1.6 mg/dl; proteinuria 9.9 g/24 h) and his diurnal arterial pressure was highly unstable. At histologic examination, lipoprotein depositions were evident within glomerular capillaries. Despite his lipoprotein profile was in normal range, the patient was treated with LDL apheresis (HELP system) every two weeks.

**Results.** After the first three LDL apheresis treatment - without side effects - blood pressure control was improved, later stabilized with a consistent reduction of ACE inhibitors/sartanic and the diuretics withdrawal. The renal function progressively recovered (eGFR normalized) with a significant proteinuria reduction (0.6 g/24 h) and parallel increase in total serum protein. Body weight stabilized to 66.8 kg from the pre-treatment weight of 76.1 kg.

**Conclusions.** The dramatic improvement of renal function, together with blood pressure control and without major lipid abnormalities, let to hypothesize a main effect of LDL apheresis on renal endothelial function.

**PLASMA CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) AND CAROTID INTIMA-MEDIA THICKNESS IN EUROPEAN INDIVIDUALS AT HIGH CARDIOVASCULAR RISK**

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Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that is bound mainly to HDL particles and mediates the transfer of neutral lipids (CE and TG) between HDL and apoB-containing lipoproteins (LDL and VLDL). Inhibition of CETP is considered a potential approach to treat dyslipidemia; however, discussion regarding CETP inhibition as a potential strategy for prevention of atherosclerosis has been controversial. As the role of CETP in the atherogenic process is still not fully clarified, we studied the association of CETP concentration with intima-media thickness of the carotid artery (IMT) in subjects with high cardiovascular risk and free of any pharmacological treatments.

The plasma CETP concentration was measured by an immunoenzymatic assay developed in our laboratory in a subgroup (n=540) of subjects enrolled in the multicenter, longitudinal, observational IMPROVE study. Mean and maximum IMT of the whole carotid tree was measured by B-mode ultrasonography in all subjects. In the entire cohort, CETP quartiles were not associated with carotid mean and maximum IMT (P for trend 0.64 and 0.66, respectively), also after adjustment for age, gender, HDL-C, and triglycerides. In the whole group of examined subjects, plasma CETP concentration correlated positively with HDL-C (P< 0.0001). The present findings show that in European individuals at high cardiovascular risk CETP concentration is not a major determinant of carotid IMT.

**MEDITERRANEAN DIET SCORE FOR PREVENTING TOTAL AND CARDIOVASCULAR MORTALITY - IS IT POSSIBLE TO DETERMINE THE “IDEAL” CONSUMPTION FOR EACH FOOD GROUP?**

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**Objective.** We have previously demonstrated that a greater adherence to Mediterranean diet reduces the risk of overall and cardiovascular mortality. However, the clinical application of the adherence score to Mediterranean diet is difficult since cut-off values for the computation of this score are extremely different among the cohorts. Aim of this study was to systematically review all the literature in order to obtain one single cut-off value of consumption for each food group composing the adherence score.

**Methods.** Cohort prospective studies investigating adherence to Mediterranean diet and health outcomes were searched through all the electronic databases, and cut-off values of food groups used to compute the adherence score were obtained from each single study.

**Results.** Twenty-seven cohort studies with an overall population of 2,876,762 participants were identified. The cumulative analysis under a random-effects model showed that 2-point increase of adherence score to Mediterranean diet confirmed to determine a 9%-reduction of overall mortality (HR: 0.91; 95%CI 0.894-0.93), 11%-reduced risk of cardiovascular disease (HR: 0.88; 95%CI 0.86-0.92) and a 5%-reduction of neoplastic disease (HR: 0.95; 95%CI 0.93-0.97). In order to estimate the “ideal” value of consumption for each food group composing the adherence score to Mediterranean diet we calculated the weighted average values of consumption according to sample size and gender. This analysis allowed us to create the “ideal” adherence score in terms of primary prevention of overall and cardiovascular mortality.

**Conclusion.** We were able to quantify, through an updated review with meta-analysis, the exact consumption of each food group composing the adherence score to Mediterranean diet in terms of a primary prevention of overall and cardiovascular mortality. This could help the application of such tool in the clinical practice.
IMPACT OF A REPLACEMENT DIET WITH KHORASAN WHEAT (KAMUT®) PRODUCTS ON CARDIOVASCULAR RISK FACTORS: CROSS-OVER DIETARY INTERVENTION STUDY

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Background and aim. Khorasan wheat (Kamut®) is an ancient grain with supposed beneficial effects for health. The aim of this study was to examine the effect of a replacement diet with grain products made from Kamut® wheat on cardiovascular risk factors, and redox status compared to a whole-grain durum wheat-diet.

Materials and Methods. We conducted a randomized, single-blind crossover trial with 2 intervention phases among 22 healthy subjects (14 F; 8 M). The trial participants were assigned to consume grain products (bread, pasta, and crackers) made from Kamut® wheat or whole-grain durum wheat during one of the 8-week phases. Blood analyses were performed at the beginning and at the end of each phase of intervention.

Results. At a general linear model for repeated measurements adjusted for several confounders, subjects who consumed Kamut® products showed a significant reduction of cardiovascular risk factors such as total cholesterol (Pre: 210.4 (192.8-227.9) mg/dL vs. Post: 201.9 (185.4-218.4) mg/dL; p=0.03), LDL cholesterol (Pre: 125.5 (110.3-140.7) mg/dL vs. Post: 115.6 (103.7-127.6) mg/dL; p=0.02), and blood glucose (Pre: 81.1 (77.3-84.9) mg/dL vs. Post: 78.1 (75.5-80.7) mg/dL; p=0.04), whereas no similar results in the phase of intervention when subjects consumed whole-grain products made from durum wheat were observed. Similarly plasma redox status resulted to be significantly improved during the Kamut® phase, as observed by reduced levels of TBARS (Pre: 0.79 (0.63-0.95) nmol/mL vs. Post: 0.62 (0.52-0.72) nmol/mL; p=0.04) and protein carbonyl content (Pre: 0.91 (0.74-1.03) nmol/mL vs. Post: 0.75 (0.63-0.87); p=0.03). Moreover, replacement diet with Kamut® brand wheat products determined a significant increase in serum potassium and magnesium with respect to intervention with whole-grain durum wheat products, which determined no significant modifications of these parameters.

Conclusions. Our data suggest that a replacement diet with grain products made from Kamut® wheat is effective in reducing cardiovascular risk factors, including lipid parameters, and markers of redox status.

IDENTIFICATION OF A NOVEL ANGPTL3 SPlicing MUTATION ASSOCIATED TO SEVERE HYPOBETALIPOPROTEINEMIA

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Introduction. Primary hypobetalipoproteinemia (pHBL) is a monogenic heterogeneous condition inherited as a dominant or recessive trait characterized by total cholesterol (TC) and/or LDL cholesterol (LDL-C) and/or apolipoprotein B (APOB) levels below the 5th percentile of the reference population. Heterozygous APOB gene mutations are responsible for the majority of the dominant pHBL causing the familial hypobetalipoproteinemia (FHBL). Loss-of-function mutations in the PCSK9 gene also cause FHBL. Familial combined hypolipidemia is a recently discovered dyslipidemic phenotype characterized by low levels of TC, triglycerides (TG), LDL-C, and high-density lipoprotein cholesterol (HDL-C). The genetic cause of familial combined hypolipidemia has been attributed to mutations in the ANGPTL3 gene.

Methods and Results. In this report we describe a case of a young men with severe hypolipidemia characterized by low levels of total cholesterol, triglycerides and HDL (54 mg/dL, 26 mg/dL, 17 mg/dL respectively). The proband’s mother and father showed normal plasma lipid values suggesting a recessive mode of inheritance of the phenotype.

In order to identify the molecular defects the analysis of MTP and SARA2 genes was carried out and no mutations were identified in both genes. We extended our analysis to the other known genes responsible for pHBL and we were able to identify in the ANGPTL3 gene a novel splicing mutation (InsT+3, IVS5) in heterozygosity. Both parents were carriers of the same mutation in the same exon. The proband’s mother and father showed normal plasma lipid values suggesting a recessive mode of inheritance.

Conclusion. We describe a clinical case with severe hypolipidemia with a recessive mode of inheritance carrying a novel mutation in Intron 5 of ANGPTL3. The mode of inheritance and the clinical implications of familial combined hypolipidemia need to be further characterized.

THE INTRACELLULAR CHOLESTEROL CONTENT MODULATES THE ENDOCANNABINOID SYSTEM ACTIVITY IN FOAM CELLS

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A growing body of evidence suggests that the endocannabinoid system might play a critical role in the pathogenesis of atherosclerosis. In this study we investigated the expression of endo-
cannabinoid receptors and the effect of endocannabinoid system stimulation on cholesterol metabolism in foam cells. Treatment of J774 and RAW 264.7 cell lines and primary mouse peritoneal macrophages with 100 μg/mL acetylated LDL (AcLDL) increased the intracellular total cholesterol levels and significantly increased the expression of the CB1 receptor while reducing the anandamide degrading enzyme FAAH, whereas CB2 receptor levels were not affected. Incubation of RAW 264.7 cells with 5 μM WIN 55,212-2, a CB1 and CB2 receptors full agonist, resulted in significantly increased CD36 expression, as assessed by flow cytometry, but this effect is abrogated in presence of AcLDL and co-incubation of WIN 55,212-2 with AcLDL does not modify the intracellular total cholesterol levels compared to AcLDL treated cells. Q-PCR analysis showed significantly decreased ABCA1 but similarly to what observed in RAW 264.7 gene expression in RAW 264.7 cells incubated with WIN 55,212-2. Further analysis in AcLDL loaded primary mouse peritoneal macrophages showed that 5 μM WIN 55,212-2 treatment specifically reduces the cholesterol efflux to ApoA1 (40%, p<0.03) whereas the cholesterol efflux to HDL3 is not altered. Taken together our data show that CB1 receptor expression is positively regulated by intracellular cholesterol and that endocannabinoid system stimulation does not affect modified LDL uptake but reduces ABCA1 mediated cholesterol efflux in foam cells.

ANALISI MUTAZIONALE DEI GENI APOA1 E LPL NELLA SINDROME DA BASSE HDL IN ETA' PEDIATRICA

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Le dislipidemie aterogene sono alterazioni dei livelli dei lipidi nel plasma altamente correlate a rischio cardiovascolare. La sindrome da basse HDL rientra in questo quadro di manifestazioni con il coinvolgimento di numerosi geni e dunque complessa analisi mutazionale.

Su una casistica pediatrica di 84 bambini con valori di colesterolo HDL <40 mg/dl abbiamo condotto l’analisi mutazionale per sequenziamento di 2 geni candidati, quello per la lipoproteïnlimpasi (LPL) e quello per l’apoproteina A1 (APOA1). L’analisi mutazionale è stata condotta attraverso una piattaforma strumentale automatizzata in formato 96-well comprendente il sistema robotico Microlab Starlet (Hamilton), l’analizzatore genetico ABI PRISM 3130xl (AppliedBiosystem) e il software SeqScape (Applied Biosystem) per l’analisi dei dati. Come popolazione di controllo è stata utilizzata quella del database SNP.

Nel gene LPL sono state identificate le seguenti variazioni di sequenza: 1 al 5’UTR (281T>G), 9 esoniche (N291S, L365V, D9N, S45N (nuova), R89Q (nuova), V106V, E118E, T106T, S474X) e 15 introniche. Le variazioni N291S, L365V, -281T>G e D9N (ad elevata frequenza complessiva nella popolazione analizzata: 0.233, le prime 2 p<0.01 rispetto ai controlli), sono state associate a fenotipi iperlipidemici con bassi livelli di HDL. La S474X (a bassa frequenza nella popolazione analizzata: 0.071, rispetto ai controlli 0.120, p=0.058), risulta associata ad un’aumentata attività della LPL, con bassi valori della LPL e basse quantità di trigliceridi nel siero e diminuito rischio cardiovascolare.

Nel gene APOA1 sono state identificate le seguenti variazioni di sequenza: 1 nel promotore (-75G>A), 21 introniche (9 delle quali muove) e 1 esonica (A188S). L’unica funzionalmente descritta è la -75G>A (ad elevata frequenza nella popolazione analizzata: 0.161, p<0.05 rispetto ai controlli) che sembra influenzare il tasso di trascrizione del gene ed è associata a bassi valori di HDL.

L’identificazione delle lesioni molecolari nei geni candidati e la loro caratterizzazione risultano indispensabili al miglioramento dell’abilità diagnostica, prognostica e terapeutica di tale sindrome.

GEPHERETIO EPLAOGH ThESIS TISSE N EAT IDs TES LIPID-SSENSING NUCLEAR RECEPTOR PATHWAYS IN CORONARY ARTERY DISEASE

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Background. Epicardial adipose tissue (EAT) is the energetic reservoir of the heart, with a thermogenic and secretory potential. Obesity and Metabolic Syndrome (MS) induce EAT enlargement, inflammation, enhanced secretion of vasoactive and inflammatory modulators, events that could promote atherosclerosis. We studied whole-genome gene and miRNA profile of EAT in patients with CAD, with the aim of understanding the molecular events involving EAT in atherosclerosis, and of identifying putative targets for CAD prevention.

Material and Methods. We enrolled 23 male subjects, 10 nonmetabolic patients, with no evidence of CAD, undergoing cardiac valve-replacement (controls), and 13 metabolic CAD patients undergoing coronary artery bypass graft surgery (CABG). We collected clinical and biochemical data, serum, and EAT for the study of gene and miRNA expression profile.

Results. When compared to controls, CABG patients were characterized by increased blood pressure, heart rate, HbA1c, ESR, CRP, and fibrinogen, while HDL-c was decreased (p<0.05). Although not significantly, BMI, abdominal circumference, fasting glycaemia, insulin resistance, and triglycerides were increased. Up to 7000 genes were expressed in EAT, with 57 significantly up-regulated and 88 down-regulated genes in CABG group; of the 580 miRNAs expressed in EAT, 16 were significantly up-regulated and 16 were down-regulated in the CABG group (cut-off 1.2 folds; adjusted p-value<0.05). We dissected the biological networks differentially expressed in CAD, and we found a strong up-regulation of inflammatory pathways and cytokine production, and a down-regulation of lipid metabolism, mitochondrial function, and lipid-sensing nuclear receptor transcriptional activity. We also found intriguing correlations between the differentially regulated miRNAs, genes, and clinical variables.

Conclusions. EAT displays a distinctive molecular signature directly connected to the pathophysiology of atherosclerosis. EAT transcriptome is a source of putative targets for the prevention of atherosclerosis in MS.
CLINICAL, MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF TWO NOVEL SPlicing MUTATIONS ASSOCIATED TO COMPOUND HETEROZYGOUS FHBL

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Introduction. Primary hypobetalipoproteinemia (pHBL) is a monogenic heterogeneous condition characterized by total cholesterol (TC) and/or LDL cholesterol (LDL-C) levels below the 5th percentile of the reference population. Heterozygous APOB gene mutations are responsible for the majority of the dominant pHBL causing the familial hypobetalipoproteinemia (FHLB). The clinical phenotype of heterozygous FHBL is usually mild. The homozygous or compound heterozygous APOB mutations are in some cases responsible for a more severe biochemical and clinical phenotype, similar to the abetalipoproteinemia (ABL) due to homozygous mutations in the MTP gene, characterized by intestinal malabsorption, pigmentary retinal degeneration, ataxic neuropathy, and almost undetectable levels of LDL-C and APOB.

Methods and Results. In this report we describe the clinical and molecular characterization of a child with severe hypolipidemia and a clinical phenotype suggesting the clinical diagnosis of ABL. Parents serum total cholesterol (TC) and LDL cholesterol (LDL-C) levels however were below the 5th percentile thus suggesting that the proband could have been homozygous or compound heterozygous FHBL. Western blot analysis showed the presence of two truncated forms of apoB protein of different molecular weight in the serum of the proband; a single truncated form was then observed in the mother on in the father. The direct sequence the apoB gene in the proband allowed to identify two novel mutations: a) a mutation of the donor splice site of intron 23 of the APOB gene (c.3824+1G>A) and b) a mutation of the acceptor site of intron 23 of of the apoB gene (c.3825+1G>C). The parents were heterozygous carriers. Splicing mechanisms of both mutations were further characterized by minigene assay in vitro. The results of the present study confirm the alterations of the lipid/lipoprotein profile observed in dialysis patients, demonstrate the alteration of the cholesterol esterification process and HDL subclass distribution, and suggest that the observed alterations are more pronounced in HD patients.

CARDIOMETABOLIC PHARMACY, AN ALLIANCE BETWEEN MEDICAL DOCTORS AND PHARMACISTS TO IMPROVE THE PHARMACOLOGICAL ADHERENCE AND THE CARDIOVASCULAR PREVENTION

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A poor control of risk factors is often due to lack of patient compliance with prescribed medical therapy. In order to improve cardiovascular prevention strategies, the CNR has created a network between doctors and pharmacists, the “Cardio-metabolic pharmacy”. To participate in the study, pharmacists were trained by CNR on cardiovascular prevention. Subsequently, through computer-based patient records, pharmacists reported the values of the patient’s main cardiovascular risk factors as well as current therapy. Results were sent to CNR through tele-medicine systems. Within 48 to 72 hours, the medical team replied with a calculated 10-year cardiovascular risk to the pharmacy, together with comments and advice on nutrition and therapy. The pharmacist explained this report to the patients, and informed their medical doctor. Subsequently, 300 patients were divided randomly in two groups: the “intervention” group (n. 200 of participants) had a follow-up of four visits; the “control” group (n. 100) received only the final visit. In the intervention group 94,2% of the patients maintained adherence to the drug therapy against 68,7% in the control group (p<0,001). This result explains the better risk factors profile and 10-year cardiovascular risk in the intervention group (baseline 12,6, final 10,8,

Background. Chronic kidney disease is associated with premature atherosclerosis and increased cardiovascular morbidity and mortality. One of the major cardiovascular risk factors is represented by dyslipidemia.

Methods and Results. In the present study, 43 dialysis patients were enrolled: 21 on Hemodialysis (HD) and 22 on Peritoneal dialysis (PD). The two groups were matched for age and gender. In addition to plasma lipids and lipoproteins, LCAT concentration, LCAT activity and CER (cholesterol esterification rate) were determined. HDL subpopulation distribution according to protein composition, size and shape, was also analysed. A control group (CG), also matched for age and gender, was recruited. Plasma HDL-C, ApoA1, and ApoAII levels were significantly reduced in patients on dialysis, with no differences between HD and PD. As expected, marked hypertriglyceridemia associated with elevated plasma VLDL concentration was observed. The FC/TC (free cholesterol/total cholesterol) ratio was significantly increased and plasma LCAT concentration decreased in patients on dialysis compared to the CG, again with no difference between HD and PD. On the contrary, LCAT activity was significantly decreased only in patients on HD. HDL subclass distribution was shifted towards small, LpA-I particles; these changes were more pronounced in HD patients.

Conclusions. The results of the present study confirm the alterations of the lipid/lipoprotein profile observed in dialysis patients, demonstrate the alteration of the cholesterol esterification process and HDL subclass distribution, and suggest that the observed alterations are more pronounced in HD patients.
G. Russi, M. Fonda, L. Cattin

Possibly explain the increased cardiovascular morbidity and mortality observed in patients treated with CsA. The objective of the present work was to investigate the mechanisms accounting for this observation. Methods: CsA interference on the first step of RCT, cholesterol efflux from macrophages, was evaluated with an in vitro assay, where murine peritoneal macrophages were incubated with increasing concentrations of the drug. Cell capacity to release cholesterol into cell medium was quantified with a radioisotope-based method. CsA influence on the last step of RCT, sterol efflux in the intestinal lumen, was assessed by in vivo and in vitro approaches. In the former, the gene and protein expression of ATP Binding Cassette G5 (ABCG5) and ABCG8, was quantified in the liver of C57BL/6 mice treated with CsA 50 mg/kg/d for 7 days. In the latter, ABCG5- and ABCG8-expressing human colon carcinoma (CaCO2) cells were incubated with CsA and the release of radiolabeled cholesterol from the apical membrane into the extracellular medium was measured. Results: CsA 0.1-10 μM did not affect macrophage capacity to release cholesterol to murine plasma. Mice treated with CsA showed higher amount of Abcg5 and Abcg8 (mean of fold increase ±s.d.: 2.61±0.87 versus 4.63±2.05; p<0.05 and 5.13±2.23 versus 7.62±2.53 in vehicle and CsA-treated mice respectively) mRNA, but no differences in protein expression. However, CsA significantly inhibited sterol efflux from CaCO2 cells (% cpm released into cell media ±s.d.: 6.49±1.42 versus 4.04±0.34; p<0.05 in cells untreated or treated with CsA respectively). Conclusions: We demonstrated that CsA impairs the activity of ABCG5 and ABCG8 in vitro, without reducing their expression in vivo. This effect could result in the impairment of cholesterol elimination from the body and the inhibition of macrophage RCT.

THE IMMUNOSUPPRESSIVE DRUG CYCLOSPORINE A IMPAIRS THE REVERSE CHOLESTEROL TRANSPORT IN VIVO BY REDUCING STEROL FECAL EXCRETION

I. Zanotti, G. Lusardi, D. Greco, F. Poti, F. Bernini

We previously demonstrated that a short term administration of CsA to mice impaired the antiatherosclerotic process of the macrophage reverse cholesterol transport (RCT). This effect could possibly explain the increased cardiovascular morbidity and mortality observed in patients treated with CsA. The objective of the present work was to investigate the mechanisms accounting for this observation. Methods: CsA interference on the first step of RCT, cholesterol efflux from macrophages, was evaluated with an in vitro assay, where murine peritoneal macrophages were incubated with increasing concentrations of the drug. Cell capacity to release cholesterol into cell medium was quantified with a radioisotope-based method. CsA influence on the last step of RCT, sterol efflux in the intestinal lumen, was assessed by in vivo and in vitro approaches. In the former, the gene and protein expression of ATP Binding Cassette G5 (ABCG5) and ABCG8, was quantified in the liver of C57BL/6 mice treated with CsA 50 mg/kg/d for 7 days. In the latter, ABCG5- and ABCG8-expressing human colon carcinoma (CaCO2) cells were incubated with CsA and the release of radiolabeled cholesterol from the apical membrane into the extracellular medium was measured. Results: CsA 0.1-10 μM did not affect macrophage capacity to release cholesterol to murine plasma. Mice treated with CsA showed higher amount of Abcg5 and Abcg8 (mean of fold increase ±s.d.: 2.61±0.87 versus 4.63±2.05; p<0.05 and 5.13±2.23 versus 7.62±2.53 in vehicle and CsA-treated mice respectively) mRNA, but no differences in protein expression. However, CsA significantly inhibited sterol efflux from CaCO2 cells (% cpm released into cell media ±s.d.: 6.49±1.42 versus 4.04±0.34; p<0.05 in cells untreated or treated with CsA respectively). Conclusions: We demonstrated that CsA impairs the activity of ABCG5 and ABCG8 in vitro, without reducing their expression in vivo. This effect could result in the impairment of cholesterol elimination from the body and the inhibition of macrophage RCT.
62,59±11,19%. ICAM, VCAM, IL6, IL10 non hanno mostrato variazioni significative in acuto, tuttavia IL10 ha mostrato un sensibile aumento dopo LA (6,84±4,76 vs 11,51±9,31 pg/mL, p=0,066). PTX3 basale correlava con IL6 sia basale che dopo aferesi (rispettivamente 0,67, p=0,034 e 0,79, p=0,006). Nei 5 pazienti rivalutati al FU PTX3 pre-aferesi presentava valori ridotti rispetto al T0 (T0 1,97 - FU 0,85 ng/mL), mentre IL10 risultava più elevata (T0 6,56 - FU 16,56 pg/mL).

**Conclusioni.** Questo studio preliminare suggerisce che la riduzione di PTX3 può essere considerato come un ulteriore effetto pleiotropico della LA. Studi specifici al riguardo sono necessari per confermare questa ipotesi.

**FLOW-MEDIATED DILATION, CAROTID WALL THICKNESS AND HDL FUNCTION IN SUBJECTS WITH HYPERALPHALIPROTEINEMIA: THE HYPERALPHALIPROTEINEMIA AND ATEROSCLEROSIS (HALA) STUDY**

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Prospective observational studies show a consistent inverse relationship between serum high density lipoprotein-cholesterol (HDLc) levels and the risk of cardiovascular events in the general population. However little evidence supports HDLc benefits beyond the upper levels found in epidemiological surveys (70-75 mg/dl): data concerning the profile in subject with very-high plasma HDLc levels are limited and controversial, while drug treatment strategies that resulted in a marked increase in HDLc have not proved to be helpful. Hyperalphalipoproteinemia (HAL) is an heterogeneous condition defined as HDLc rising above the 90th percentile of the general population, that may be related to acquired factors (some medications, liver and thyroid diseases, ethanol assumption, physical exercise) or to genetic influences. HDL arterial protection has been mainly related to reverse cholesterol transport (RCT) promotion; HDL particles heterogeneity may modulate functional capabilities and their vascular effects, partly explaining why total HDLc concentration represents a useful but deficient parameter to characterize RCT efficacy.

**Objective.** To better define the potential atheroprotective role of high HDLc plasma levels, we investigated subjects with presumptive primary HAL, measuring indexes of subclinical atherosclerosis such as flow mediated dilation (FMD) and carotid intima-media thickness (cIMT), and evaluating HDLc functionality by its capacity to promote cholesterol efflux (CEC). Methods: 20 subjects with HDLc >85 mg/dl and 20 with HDLc levels within normal range were tested for FMD and CEC through aqueous diffusion- (AD), SR-BI-, ABCG1- and ABCA1-mediated CEC using apolipoproteinB-depleted serum.

**Results.** FMD and cIMT did not correlate with HDLc and were comparable in case and control subjects. HDL PD- and SR-BI-mediated CEC were higher in control subjects (6,41%±0,77 vs 5,16%±0,72, p<0,001, and 4,67%±0,95 vs. 2,99%±0,58, p<0,001 respectively). After normalizing efflux values for the HDLc levels, serum HDL PD-mediated CEC turned greater in control subjects (0,095%±0,021 vs. 0,067%±0,009, p<0,001), while no difference was detected in SR-BI mediated CEC. HDL ABCG1-CEC was similar in both groups, but after normalizing efflux for HDLc levels, it was higher in normal subjects (0,078%±0,005 vs 0,05%±0,0024; p<0,001). HDL ABCA1-CEC displayed pattern similar as compared to ABCG1. Small HDL particles were higher in normal subjects (19,95%±0,99 vs 16,02%±0,87; p<0,01) while large HDL particles were higher in HAL subjects (42,29%±1,44 vs 33,49%±1,43; p<0,001).

**Conclusions.** HDL AD- and SR-BI-CEC pattern are related to HDLc levels; however, correction for HDLc levels of AD-CEC caused significantly higher CEC in control subjects, reversing previous results. We may speculate that the cholesterol/phospholipid HDL molar ratio in HAL subjects, that are characterized by larger HDL particles, cause a concentration gradient, driving passive cholesterol flux, weaker than in control subjects. ABCA1- and ABCG1-CEC are metrics of HDL functionality: indeed, normalizing for HDLc, HDL from normal subjects display an higher efficiency through ABCG1 and more moderately ABCA1. Such enhanced HDL functionality, probably reflecting higher percentage of smaller HDL in normal subjects, might explain, at least in part, the lack of difference in subclinical atherosclerosis markers in spite of large differences in HDLc concentration between the two investigated groups.
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