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HDL-C PROFILE AND BMI IN DYSLIPIDEMIC CHILDREN

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Objective. The relationship between lipoprotein profile and vascular damage is well recognized. Furthermore HDL-C represent an important biochemical marker related to overweight/obesity and to CVD risk in adults.
Aim. Of this study was to evaluate the correlation between lipid biochemical markers (TC, LDL-C, HDL-C, apoB and apoA-I levels) and the staturo-ponderal growth in a cohort of dyslipidemic children. Study design. A cohort of 231 patients (aged 10.9±3.7 years) was enrolled. Seventy-six children resulted affected by FH, 68 by FCHL and 87 by dominant Hypercholesterolemia. Demographic data, medical history, physical examination and two generations family history of cardiovascular events were evaluated in the entire cohort. Children were submitted to biochemical analysis concerning TC, HDL-C, TG, apoB and apoA-I by standard methods, after an overnight fasting. Statistical analysis: Pearson correlation analysis was performed.
Results. HDL-C resulted negatively correlated to BMI (r=-0.300, p<0.001), TG ApoB/ApoA1 (p<0.001) and positively correlated to TC and ApoA1 (p<0.001). Furthermore BMI resulted positively correlated to age and TG (p<0.001) and negatively to ApoA1. (p=0.048).
Conclusion. The present findings indicate that HDL-C represent a significant parameter to check in children particularly when overweight occur.

EFFECT OF THE CHOLESTEROL-LOWERING COMPOUND BERBERINE ON ABCA1- AND ABCG1-MEDIATED CHOLESTEROL EFFLUX FROM MACROPHAGES

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Objective. Cholesterol efflux is the first step of reverse cholesterol transport that protects peripheral cells from excess cholesterol accumulation. Efflux can occur by passive and SR-BI-facilitated diffusion; ABCA1 and ABCG1 mediated processes. Berberine (BBR), a cholesterol-lowering alkaloid, has several antiatherosclerotic properties; however, it was also shown to promote foam cell formation.
Aim. The aim of our study was to evaluate the effect of BBR on intracellular cholesterol metabolism, focusing on ABCA1 and ABCG1-cholesterol efflux in mouse peritoneal macrophages (MPM). To this aim cells were labeled with [3H]-cholesterol in the presence or absence of acLDL for 24 h. Then MPM were equilibrated in 0.2% BSA-containing medium in the presence or absence of LXR-RXR agonists. Cholesterol efflux to apolipoprotein A-I (apo A-I) or HDL2 was measured by the release of [3H]-cholesterol into the medium and calculated as percentage of total cell radioactivity. In acLDL-loaded MPM BBR 1μM reduced ABCA1-cholesterol efflux to apoA-I by 51% ± 9.2, whereas ABCG1 efflux to HDL2 was reduced by about 100%. BBR did not affect cholesterol uptake (88.31 ± 7.64 μg/mg protein versus 91.41 ± 3.25 μg/mg protein without or with BBR respectively). As measured by TLC, the percentage of intracellular cholesteryl ester (CE) was unchanged after BBR treatment (47.89% ± 3.41 versus 45.9% ± 2.53 without or with BBR respectively). This was confirmed by the observation that BBR had an inhibitory effect on ABCA1- efflux to apoA-I (55% ± 1.4) also in the presence of an ACAT inhibitor. In LXR-RXR treated MPM, the inhibitory effect of BBR was more modest either on ABCA1-efflux to Apo A-I (22% ± 6.4), and on ABCG1-efflux to HDL2 (63% ± 9.9). In conclusion BBR reduced ABCA1 and ABCG1-efflux in acLDL-loaded MPM, without affecting cholesterol uptake and percentage of CE. In LXR-RXR-treated MPM, the inhibitory effect of BBR was more modest. Our preliminary results suggested that BBR, despite its cholesterol-lowering effect may have a potential pro-atherosclerotic activity reducing both ABCA1 and ABCG1-efflux.

CIRCULATING REGULATORY T CELLS IN CAROTID AND CORONARY ATHEROSCLEROTIC DISEASES

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Background. Regulatory T (Treg) cells have been related to the control of experimental atherosclerosis. The analysis of Treg-cell levels in patients with coronary atherosclerosis however, resulted in mixed observations. The aim of the present study was to investigate the levels of circulating Treg cells and their homing properties together with levels of activated T cells, in the initial and late stage of plaque development in carotid and coronary arteries.
Methods and Results. We studied two distinct populations: a) 113 subjects, selected from the general population (carotid study), in which we measured the intima-media thickness (IMT) of the common carotid artery, as a surrogate marker of initial atherosclerosis and b) 75 controls and 125 patients with coronary artery disease (CAD) (coronary study): 36 with chronic stable angina (CSA), 50 with Non-ST-elevation Acute Coronary Syndrome (NSTACS), 39 with ST-Elevation acute Myocardial Infarction (STEMI). Treg-cell levels were evaluated by flow cytometry (Treg cells identified as CD3+CD4dimCD25highCD127low), mRNA expression of FoxP3 or Treg-associated cytokine interleukin (IL)-10. In the carotid study no correlation was observed between Treg-cell levels and IMT. When the presence of Treg cells was investigated in a subgroup of patients (n=65) where prospective data on six-year IMT progression were available, no differences were observed comparing rapid versus slow IMT progressors. In the coronary group the percentage of Treg cells was increased in STEMI while reduced in NSTACS (P<0.001 for both vs controls and vs CSA group) and was not altered in CSA patients. Treg cells were significantly increased in both NSTACS and STEMI groups after 55 days of follow-up. Serum IL-10 levels showed quite similar behavior of Treg cells in the coronary study. Activated T cells were increased in NSTACS and STEMI patients with a significant proinflammatory imbalance in NSTACS patients. Furthermore we did not find significant changes in CCR5+Treg subsets in CAD. Conclusions- We did not find significant changes in circulating Treg-cell levels in rela-

tion to extent or progression of human atherosclerotic disease at carotid and coronary sites. A minimal perturbation of Treg-cell subset could be associated with plaque destabilization. The results suggest that determination of circulating Treg-cell level is not useful indicator of the extent or severity of atherosclerosis.

BIOLOGICAL MARKERS OF ASPIRIN RESISTANCE: THE RELEVANCE OF OXIDATIVE STRESS

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Background and aims. Despite aspirin reduces the odds of thrombotic events in high-risk patients via its antiplatelet effect, a considerable proportion of patients treated with aspirin do not show platelet inhibition. The mechanisms underlying "aspirin resistance" are multifactorial. Aim of this study was to compare platelet function and circulating biomarkers of inflammation, endothelial dysfunction, glycemic and lipidic control, insulin resistance and oxidative stress in patients on chronic aspirin-treatment who resulted "sensitive" or "resistant" to aspirin according to standardized tests.
Materials and Methods. We studied 56 patients on chronic aspirin therapy (100 mg/day) owing to the presence of severe cardiovascular risk factors and/or previous cardiovascular events: M/F 33/23; age: 64.5±0.82 years; body mass index: 27.7±0.5 kg/m2. Platelet sensitivity to aspirin was evaluated in platelet-rich plasma (PRP) by determining by Born's method Maximal Aggregation (MA) in response to Sodium Arachidonate (NaAA): patients were defined as "aspirin resistant" when MA in response to NaAA was greater than 20%. In aspirin sensitive and resistant patients, we evaluated: i)age, ii)gender, iii)BMI, iv) Diastolic and Systolic Blood Pressure; v)markers of glycaemic control and of insulin resistance (HbA1c, fasting glucose, fasting insulin, C-Peptide, HOMA-IR), vi)markers of lipidic control (Total, HDL and LDL-cholesterol, Triglycerides), vii) markers of platelet function (platelet aggregation by Born's method induced by 1 mmol/l NaAA, 10 µmol/l ADP, 4 µmol/l epinephrine, 4 mg/l Collagen); TXB2 concentrations in both resting PRP and at the end of each agonist-induced aggregation (EIA); sCD-40 Ligand; P-Selectin) viii) inflammatory markers (serum levels of the pro- and anti-inflammatory and atherogenetic cytokines and inflammatory markers, i.e., IL-1ra,-1b,-4,-5,-6,-7,-8,-9,-12,-13, TNF alpha and CRP, ix) adhesion molecules (sICAM, sVCAM), x)chemokines (MCP-1, RANTES, MIP-1b, IP-10), xi)growth factors (VEGF, PDGF), and xi) biomarkers of in vivo oxidative stress 8-OH-2'-deoxyguanosine (8-OHdG) and oxidized LDL (ELISA).
Results. Aspirin resistant patients identified by MA to NaAA were 7/56 (12.5%). Compared with the aspirin sensitive-ones, they presented:

1) greater baseline platelet responses to agonists: in particular, MA was: 30.4±3.0% vs 6.4±0.5% ($p<0.0001$) in response to NaAA; 100.3±5.9% vs 67.7±4.8% ($p<0.015$) in response to ADP; 48.1±6.9% vs 24.7±3.0% ($p<0.007$) in response epinephrine; 49.6±2.5% vs 29.8±3.8% ($p<0.05$) in response to Collagen, but similar concentrations of TXB2 in both resting status (503.0±111.3 vs 403.0±25.0 pg/ml/min, ns) and after stimulation with each agonist (in particular, after NaAA-induced aggregation: 2006.0±245.4 vs 2816.0±685.4, ns);
2) reduced circulating levels of the anti-inflammatory cytokines IL-4 (0.47±0.03 vs 0.86±0.05 pg/ml; $p<0.002$) and IL-9 (14.33±2.56 vs 27.2±3.79 pg/ml; $p<0.05$);
3) increased levels of 8-OHdg (84.8±8.0 vs 31.29±15.47 ng/ml, $p<0.01$).

No significant changes has been observed in the numerous other parameters mentioned above.
Conclusions. Aspirin-resistant subjects differ from the aspirin-sensitive-ones for some markers of platelet function, but not for platelet thromboxane synthesis, as already described in Literature. Furthermore, they show reduced concentrations of some anti-inflammatory markers and an increased oxidative stress. In conclusion, these results suggest a link between "in vivo" oxidative stress and aspirin resistance.

NITRIC OXIDE AND ANTIOXIDANT HYBRIDS AS A NEW POSSIBLE APPROACH IN THE TREATMENT OF ATHEROSCLEROSIS. EVIDENCE IN SMOOTH MUSCLE CELLS

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Atherosclerosis is a multifactorial disease: LDL oxidation, nitric oxide unbalance and smooth muscle cell (SMC) proliferation play pivotal roles. To design new antiatherosclerotic molecules, we synthesized differently substituted furoxans (at positions 3/4 of the heteroring) able in relaxing rat aorta strips. The involvement of NO was evident by the inhibitory effect of ODQ, a well-known inhibitor of guanilate ciclase. Then, we joined furoxans with different antioxidants to obtain NO-donor and antioxidant hybrids.
These compounds, albeit with different potency, inhibited SMC proliferation. To understand the antiproliferative mechanism of these hybrids, we split them in native pharmacophores.
We found that furoxans (but not the antioxidant moieties), decreased SMC proliferation. Blocking the position 4 of their ring by a phenyl group, the inhibitory potency on SMC proliferation and vasodilation paralleled with the electron-attractor capacity of the group in 3.
Extending the study to 4-R, 3-Ph furoxans (groups in 3 and 4 are interchanged) and to their related furazans (unable to release NO), the 4-Ph-3-R furoxans were the most potent inhibitor of SMC proliferation, followed by 3-Ph-4-R furoxans. Furazans were not effective, supporting the fact that the opening of the ring is essential for growth-inhibition (figure).
To understand the molecular basis of this effect, we demonstrated that the mechanism is neither cGMP- nor polyamine-dependent, the two main NO-mediated pathways involved in SMC proliferation. The effect is possibly due to a thiol-mediated S-nitrosylation of a protein involved in the growth process (a mechanism proposed to explain the inhibitory activity of furoxans on thioredoxin glutathione reductase, the enzyme responsible of maintaining the redox balance in Schistosoma).
We are attempting to verify this mechanism by in vitro and ex vivo approaches and to test these hybrids in an animal model of atherosclerosis.

IN DIALYZED PATIENTS, HDL SCAVENGER ACTIVITY IS INDEPENDENT OF HDL PLASMA LEVELS

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Background. High density lipoproteins (HDL) promote cholesterol efflux (ChEf) from cells to the liver, playing a key role in cholesterol removal. HDL cellular ChEf is mediated by cellular B-I (SR-BI) and ATP-binding cassette–A1 (ABCA-1) membrane scavenger receptor. Low HDL plasma levels are a cardiovascular risk factor in patients with kidney dysfunction. Aim of this study was to address HDL plasma levels and function in dialysis patients (DP).
Material and Methods. 19 DP (6 M, 13 F) were studied and 10 healthy subjects age and sex matched were enrolled. Plasma samples were collected form all subjects. ChEf potential was tested with both macrophages and hepatoma cells addressing either SR-BI or ABCA-1. These cells were filled in with cholesterol and tested with serum HDL of both DP and controls in the medium.
Results. HDL plasma levels were lower in DP than in controls (44±9 mg/dL vs 62±9mg/dL, p<0.001). There was no difference between SR-BI and ABCA-1 mediated ChEf controls and DP (23.8% vs 23.6%, p=n.s.; 15.8 vs 14.4 % p=n.s; respectively). Controls SR-BI mediated ChEf is significantly directly correlated with HDL plasma levels (r2=0.41 p<0.01). This correlation is lost in DP. Comparing ChEf potential in DP serum before and after the dialysis, SR-B1 mediated ChEf was increased up to 26.1% post-dialysis and returned to basal levels prior to the next dialysis. As post-dialysis HDL plasma levels are increased too, we focused the next analysis on the direct effect of HDL themselves on ChEf. In DP, ultracentrifugated post-dialysis HDL tested with machrophages were more effective in promoting SR-BI mediated ChEf, independently from HDL plasma levels.
Conclusions. In DP, HDL ChEf is preserved, in spite of their lower plasma levels, but the relationship between HDL plasma levels and the efficiency of cholesterol scavenger function is lost. Dialysis improves HDL activity independently from HDL plasma levels. We can suggest that uremic toxins alter membrane scavanger receptor function independently from HDL plasma levels.

ENDOTHELIAL MICROPARTICLES AND PROGENITOR CELLS IN PATIENTS WITH INFLAMMATORY RHEUMATIC DISEASES: THE EFFECT OF ANTI-INFLAMMATORY THERAPY

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Introduction. Rheumatoid arthritis (RA) is a multisystem disease with high rates of cardiovascular morbidity and mortality. Also, Polymyalgia rheumatica (PMR) has been associated with increased risk of atherosclerosis development. Chronic inflammation has been suggested as the possible explanation for the association between AR, PMR and premature atherosclerosis. Indeed, chronic activation of the inflammatory cascade might be responsible for endothelial injury, that is accepted to promote atherosclerosis-related diseases. Since a dysregulation of the balance between endothelial injury and repair by stem cell progenitors is believed as a novel mechanism in the pathophysiology of atherosclerosis, we investigated in patients with AR and PMR the degree of endothelial injury by measuring the number of circulating endothelial microparticles and their repair potential by endothelail progenitor cells. Moreover, the effect of anti-inflammatory treatment on the balance between endothelial injury and repair was evaluated.
Methods and Results. Twenty patients with never-treated RA, 20 with untreated PMR and 30 healthy controls were recruited for the case-control study. A subgroup of 14 RA and 16 PMR patients participated in the prospective anti-inflammatory intervention open label study. The number of circulating endothelial microparticles (CD31+/CD42-) and endothelial progenitors (CD34+/KDR+) was quantified by FACS analysis. The number of endothelial microparticles was higher in patients with either RA (676±96 n/microL) or PMR (692±49 n/microL) than in control subjects (420±39 n/microL; p<0.05 for both comparisons). Also, patients with RA and PMR had lower numbers of circulating progenitors than controls (162±41 n/mL and 180±62 n/mL vs 453±91 n/mL, p<0.05 for both comparisons). No difference in the number of endothelial microparticles and progenitors was found between AR and PMR patients. Anti-inflammatory treatment was associated with a consistent attenuation of the inflammation status, as demonstrated by C-reactive protein level reduction, in RA (from 3.2±1.0 mg/dL to 0.9±0.3 mg/dL) and in PMR patients (from 3.5±0.7 mg/dL to 0.6±0.2 mg/dL). Also a significant 29% decrease in the number of endothelial microparticles and 160% increase in the number of endothelial progenitors was observed in PMR patients, and a 60% increase in the number of endothelial progenitors in AR patients. A significant correlation between C-reactive protein and endothelial microparticles reduction was found (r=0.37, p=0.04).
Conclusions. AR and PMR are associated with a significant imbalance between endothelial injury and repair, an increased number of endothelial microparticles and a reduced count of endothelial progenitors being found in both the inflammatory diseases. Attenuation of systemic chronic activation of the inflammation cascade contributes to limit endothelial fragmentation in PMR and promote endothelial repair in both PMR and AR patients.

TUBULO-INTERSTITIAL INVOLVEMENT IN HYPERTENSIVE PATIENTS WITH METABOLIC SYNDROME

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Background. Renal resistive index (RRI) are influenced by both renal (tubulo-interstitial damage) and extra-renal (arterial stiffness and atherosclerosis) determinants. Patients with hypertension and/or diabetes mellitus (DM) show stiffened arteries and high RRI values. Recently, increased RRI were associ-

ated with metabolic syndrome (MS) in patients with DM. We investigated RRI and renal volume-to-resistive index ratio (RV/RRI) in hypertensives with MS, but with neither DM nor impair fasting glucose.
Methods. We studied 85 hypertensive patients (57±10 yrs; M/F=60/20) in chronic antihypertensive therapy. MS was diagnosed by the presence of at least two of increased waist circumference, high triglycerides and low HDL-cholesterol. RRI ≥0.70 or >95% upper confidence limit expected for the age decade were considered pathologic. Decreased RV/RRI was defined for values below the median, i.e. <176 mL*m^2/Kg. A multivariable logistic regression analysis was performed to evaluate the predictive value of MS and of its components for pathologic RRI and/or decreased RV/RRI, adjusting for age, hsCRP and diuretic therapy.
Results. Patients with MS (n=28) showed lower RV/RRI values (165±26 vs 185±27 mL*m^2/Kg, P=0.02) and a higher prevalence of decreased RV/RRI (79 vs 37%, P<0.001) compared to patients without MS (n=57). The presence of MS resulted a predictor for both pathologic RRI (crude O.R. 3.0, 95%CI 1.01-8.44, P=0.033) and decreased RV/RRI (crude O.R. 6.3, 95%CI 2.20-18.00, P=0.001), even after adjustment. Both low HDL-cholesterol and increased waist circumference resulted significant independent predictors for decreased RV/RRI.
Conclusions. In our hypertensive patients, MS was associated with increased RRI and decreased RV/RRI, despite normal glucidic metabolism. MS-related tubulo-interstitial involvement seems mediated by both low HDL-cholesterol and increased abdominal fat.

IN HYPERTENSION T-LYNPHOCYTE RENIN-ANGIOTENSIN SYSTEM IS UPREGULATED BY ANGIOTENSIN II

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Background. Low grade inflammation was reported to facilitate the development of essential hypertension and target organ damage (TOD). Recently, human T-lymphocytes were shown to be endowed with a functional active renin-angiotensin system (RAS). We investigated whether in hypertensive patients a selective AngII-driven upregulation of T-cell RAS occurs and is differently modulated in presence of low-grade inflammation.
Methods. T-lymphocytes were obtained from 21 hypertensives (I-II WHO class; 16M; 56±11 years). Low-grade inflammation was defined for hsCRP>2 mg/L. Ten healthy subjects formed the age and sex-matched control group. After T-lymphocytes isolation, mRNAs for ACE and AT1-R were quantified by RT-PCR under baseline conditions and after 10-13M AngII addition to T-cells cultures. Cell pellet and supernatant ACE activity and AT1-R cell content were measured. TOD-indexes (e.g. left ventricular mass index, LVMI) were evaluated.
Results. Both in controls and hypertensives, AngII-stimulation significantly increased ACE and AT1-R mRNA levels (P<0.05). In hypertensives, the increase was earlier and higher than controls, with the highest values in hypertensives with >2 mg/L-hsCRP. Peak AngII-induced ACE and AT1-R mRNA levels were positively related to hsCRP, systolic blood pressure and BMI at the univariate analyses. The stepwise regression analyses selected hsCRP (r=0.47) and LVMI (r=0.50) as the variables independently related to peak ACE gene expression, while BMI resulted independently related to peak AT1-R-gene expression (P<0.001 for all).
Conclusions. In hypertension, an AngII-driven activation of T-cell RAS does occur. It seems further amplified by the presence of low-grade inflammation. New potential mechanism/s for mainteinance of hypertension and TOD development could be highlighted.

AGING AND ALTERATIONS OF CHOLESTEROL HOMEOSTASIS: STUDY OF PLASMA LEVELS OF OXYSTEROLS AS METABOLIC MARKERS

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Background. The relationship between aging and cholesterol metabolism is unclear. Some epidemiological evidence suggests that serum cholesterol tends to increase in adulthood, and to decrease in the elderly (1). Knowledge on the effects of aging on the pathways regulating cholesterol homeostasis is likewise limited, even if some reports showed a reduction in bile acid synthesis (2). Aim of this study is to define age-related alterations of these metabolic pathways in humans, by analysis of serum oxysterol levels.
Methods. We analyzed serum samples from 123 adult subjects (45 M, 78 F, age range 38-78) within the epidemological M.I.Col. Study (Multicentrica Italiana Colelitiasi). We determined by GC-MS the concentrations of different oxysterols recognized as markers of the different steps of cholesterol metabolism: synthesis (lathosterol), absorption (campesterol and sitosterol), degradation to bile acid (7 alpha-hydroxy-4-cholesten-3-one).
Results. A significant correlation was detected between age and cholesterol levels. The lathosterol/cholesterol ratio was lower in elderly (age > 65) than in younger subjects (102+/-39 vs 126+/-62 microg/100 microg cholesterol, mean+/-SD; P <0.05, t test for independent data). An inverse correlation was present between lathosterol/cholesterol ratio and age. No other significant differences emerged, even if a trend towards reduced absorption markers was found in the elderly.
Conclusion. These findings suggest that a reduction in cholesterol synthesis takes place in aging. This could be due to a reduction in the metabolic demand for cholesterol, leading to down-regulation of hepatic intake mechanisms (endogenous synthesis and lipoprotein uptake) (3). This is in agreement with the observed modifications of plasma cholesterol and might involve nuclear receptors SREBPs as sensors and regulators of cholesterol homeostasis. The possible implications in terms of pharmacological management of hypercholesterolemia remain to be defined.

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LOW HDL-HIGH INFLAMMATORY MARKERS IN HEART FAILURE INDUCED BY HIGH FREQUENCY PACING IN MINIPIGS

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Background. Clinical, experimental and in vitro studies suggest a major role for high-density lipoproteins (HDL) in the vascular homeostasis regulation, not necessarily related to pro- or anti-atherosclerotic mechanisms. Low HDL, together with a pro-inflammatory state, seem to be associated with left ventricular dysfunction in the absence of coronary atherosclerotic lesions, as occurs in idiopathic dilated cardiomyopathy.
Aim. To test possible correlations between the development of non-ischemic cardiac failure and altered levels of HDL and ApoAI, inflammatory markers C3, α2-macroglubulin and ceruplasmin, in a pig model of pacing-induced dilated cardiomyopathy.
Material and Methods. 8 adult male minipigs were chronically instrumented with a pacemaker connected to the left ventricular (LV) wall. Blood samples were collected at baseline, i.e. before starting the pacing protocol, and after three weeks of pacing at 180 beats/min, when LV ejection fraction was <35% and end-diastolic pressure was >18 mmHg. Statistical analysis was performed with paired Student's t-test.
Results. After three weeks of pacing there were no significant changes in total cholesterol and triglycerides compared to baseline (57±7.50 vs 53.88±13.79 mg/dl and 23.67±9.31 vs 28.43±4.50 mg/dl respectively). Conversely, HDL and ApoAI levels were dramatically decreased (21.63±2.45 vs 9.63±3.62 mg/dl, p=0.0004, and 16.86±0.97 vs 9.76±3.41 mg/dl, p=0.002, respectively). Among the inflammatory markers, α2-macroglubulin and ceruplasmin levels were significantly increased (107.14±15.65 vs 134.35±26.04 mg/dl, p=0.0314, and 26.51±3.37 vs 36.45±5.92, p=0.0096, respectively), while C3 levels were not significantly changed (14.66±4.59 vs 17.88±8.30 mg/dl).
Conclusion. Our results suggest a novel association between development of cardiac dysfunction and decrease in circulating HDL, even in the absence of other co-morbidities and alterations of total cholesterol and triglycerides.

PROTEOMICS INVESTIGATION OF PLATELETS AFTER INHIBITION AND CARDIOVASCULAR EVENTS (APICE PROJECT)

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Dual antiplatelet therapy (aspirin and clopidogrel), aimed to inhibit platelet reactivity, is the recommended standard of care for reducing the occurrence ofmajor adverse cardiovascular events (MACE) in patients with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI) with stent implantation. A large body of evidence exists demonstrating that nonresponsiveness to clopidogrel and/or aspirin is associated with increased risk of adverse clinical events.
In the framework of the "Activity of Platelets after Inhibition and Cardiovascular Events" (APICE) project, we performed the proteomic analysis of platelet protein profiles from ACS patients undergoing PCI with stent implantation on dual antiplatelet treatment.
Total protein extracts obtained from platelets of patients with or without residual platelet reactivity [RPR - by 10μM ADP or 1mM arachidonic acid (AA)-induced platelet-rich-plasma aggregation] were analyzed at the time of the acute event. Protein content and platelet reactivity have been assessed within 24 hours from the clopidogrel loading.
We have identified more than 1000 plasma proteins. Some differentially modulated proteins among patients with or without RPR by ADP (>70%) or AA (>20%)have been observed. Interestingly, significant differences were found between patient with or without RPR and between patients with AA-RPR or ADP-RPR.
Many of the differentially expressed proteins are directly involved in adherence and activation of platelet aggregation (such as platelet/endothelial cell adhesion molecule, von Willebrand factor). Other proteins are involved in the platelet cytoskeleton organization(such as filamin A, tubulin beta 1).
In conclusion, our preliminary proteomic profiling of platelets in ACS patients on dual antiplatelet treatment identifies differences among patients with or without RPR by ADP or AA identifying different actors that might play a role in the pathological mechanisms underling AA-RPR and ADP-RPR.

HDL AND ABCA1 COOPERATE IN PREVENTING THE CHOLESTEROL-INDUCED TRANSDIFFERENTIATION OF SMOOTH MUSCLE CELLS TO MACROPHAGE-LIKE CELLS

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Macrophages accumulate cholesterol and cholesteryl esters (CE) becoming foam cells (FC). However, some FC may originate from smooth muscle cells (SMCs). Rong (PNAS 2003) showed that cholesterol-loaded SMCs rapidly assume a FC-like appearance and lose the expression of SMC markers. The aim of our study was to further characterize the transdifferentiation of SMCs to macrophages and to evaluate the role of HDL3 in modulating this process.
Aortic mouse SMCs were isolated from C57/BL6 mice and loaded with increasing concentration of free cholesterol complexed to methyl-beta-cyclodextrin. Cellular free cholesterol content increased about 5-fold, while esterified cholesterol increased 2-3-fold due to a 2.5 fold increase in the activity of the esterifying enzyme ACAT. After cholesterol loading, SMCs lost the expression of SMC markers, such as myosin heavy chain and alpha-actin (-33% and -65%, respectively), and rapidly assumed a FC appearance with Oil Red O-stainable lipid droplets. Cholesterol loading of SMC enhanced, in a concentration-dependent manner, the expression of the macrophage marker Mac-2 (almost 11-fold), and of both ABCA1 (both at the protein and mRNA levels) and ABCG1 mRNA, while the expression of ICAM-1 and VCAM-

1 was not affected. Since cholesterol loading increased the expression of ABCA1, next we tested both the effect of cholesterol loading on gene expression in SMC isolated from both wild type (WT) and ABCA1 knock-out mice (ACBA1-KO), and the capacity of HDL3 to prevent such changes.
The data show that both WT and ABCA1- KO SMCs responded to cholesterol loading in the same way observed in SMC isolated from C57/BL6 mice.
The expression of some matrix metalloproteinases (MMP) mRNA was increased also in the two cell types: MMP-13 (20 to 80-fold), MMP-3 (about 12-fold), and of the tissue inhibitor of MMP-1 (TIMP-1, 2 to 3-fold). Interestingly, in the WT SMC, HDL3 showed a "preventive" effect on gene expression modification, since the addition of HDL3 reduced both the inhibition of alpha-actin and the stimulation of MAC-2, ABCA1, ABCG1 and MMPs expression, almost to the basal values. On the contrary, in ABCA1-KO SMC, the addition of HDL3 was not able to "prevent" in any way the effect of cholesterol loading on gene expression.
These results indicate that cholesterol loaded SMCs may transform into macrophage marker-positive FC with some pro-atherogenic features. HDL3 seems to play a protective role against this process, by preventing the cholesterol-induced gene expression modification, and the presence of a functional ABCA1 is also required.

MUFA AND CARBOHYDRATE/FIBER RICH DIETS DIFFERENTLY INFLUENCE THE RELATIONSHIP BETWEEN PLASMA HS-CRP AND LIPIDS IN PATIENTS WITH TYPE 2 DIABETES

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Background and Aims. Increased levels of high-sensitivity C-reactive protein (hs-CRP) are associated with type 2 diabetes and cardiovascular diseases. The aim of our study was to evaluate whether two dietary approaches recommended for diabetes and cardiovascular prevention influence this inflammatory marker.
Methods. Twelve type 2 diabetic patients followed a diet relatively rich in carbohydrates and fiber and with a low glycemic index (CHO/fiber diet) and a diet rich in monounsaturated fat (MUFA diet) for two 4-week periods according to a randomized crossover design.
sAt the end of each period, plasma levels of hs-CRP and lipoprotein fractions (separated by discontinuous density gradient ultracentrifugation) were determined at fasting and over 6 h after a test meal with a similar composition of the corresponding diet.
Results. Plasma hs-CRP levels were not significantly different after MUFA and CHO/fiber diets at fasting (2.11±2.02 vs. 1.93±1.18 mg/L, p=0.76) and 3 h (1.98±1.99 vs. 1.90±1.21 mg/L, p=0.89) and 6 h (2.00±2.06 vs. 2.07±1.45 mg/L, p=0.91) after meal. Compared with fasting, hs-CRP levels decreased significantly after the MUFA meal but not after the CHO/fiber meal. Postprandial triglyceride-rich lipoproteins were significantly lower after the CHO/fiber than the MUFA diet. Both at fasting and postprandially, hs-CRP correlated with triglyceride in whole plasma, chylomicrons, small and large VLDL after the CHO/fiber diet but not after the MUFA diet.
Conclusions. In conclusion, a MUFA rich diet and a carbohydrate/fiber rich diet induced similar effects on plasma hs-CRP concentrations. However, these dietary approaches seem to influence hs-CRP levels through different mechanisms, i.e. direct postprandial effects by MUFA, and triglyceride-rich lipoproteins mediated effects by CHO/fiber.

CARDIOVASCULAR RISK IN TYPE 2 DIABETICS AFTER HYPOLIPIDEMIC TREATMENT

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Introduction. Control of risk factors for atherosclerosis, such as high low density lipoprotein cholesterol (LDL-C) level, has been proved to be useful in the prevention of cardiovascular events, bringing about an average 25-30% reduction of cardiovascular risk. This means an unacceptably high residual risk of 70-75%. Studies with high doses of statins demonstrated that further lowering of LDL-C is associated with an increased protection against cardiovascular disease.
On these basis, guidelines of the international scientific societies suggest that in high risk patients, such as type 2 diabetics, LDL-C must be less than 100 mg/dl and optionally less than 70 mg/dl. However, residual risk remains high even if the therapeutic goal is reached, suggesting that other risk factors may operate. Among other risk factors, low HDL-C (<40 mg/dl in male and <50 mg/dl in female) and/or high triglycerides (>150 mg/dl), which are frequently seen in diabetic population, might play an important role.
Aim. Aim of our study was to investigate in a group of type 2 diabetic patients how many of them reached the LDL-C target and were also at goal for HDL-C and/or triglycerides.
Material. The study was carried out on 401 type 2 diabetic patients (234 males and 167 females), age range: 34-82 years (mean 67,4±10,04). Mean Hba1c was 7,03±1.05%, total cholesterol 175±32,62 mg/dl, LDL-C 97,0±27,71 mg/dl, HDL-C 51,1±15,35 mg/dl and triglycerides 134,8±65,0.
Results. Results Of the 401 patients, 234 were on hypolipidemic drugs (207 on statins, 4 on simvastatin plus ezetimibe, 1 on statin plus fenofibrate and 22 on fibrates). Of them, only 61 patients (26,1%) had LDL-C <70 mg/dl, 84 (35,9%) had LDL-C between 70-99 mg/dl, 72 (30,8%) between 100-129 mg/dl and 17 (7,3%) >=130 mg/dl, 77 (33%) had HDL-C <40 mg/dl (males) or <50 mg/d (females) and 86 (37%) had serum triglycerides >150 mg/dl. Of 61 patients with LDL-C <70 mg/dl, 31 (51%) had low HDL-C and/ or high serum triglycerides. Males with LDL cholesterol <70 mg/dL N % HDL-C <40 mg/dl and/or TG >150 mg/dl 16 43,2% HDL-C >40 mg/dl and/or TG <150 mg/dl 21 56.8% Females with LDL cholesterol <70 mg/dL HDL-C <50 mg/dl and/or TG >150 mg/dl 15 62,5% HDL-C >50 mg/dl and/or TG <150 mg/dl 9 37,5%.
Conclusion In our series of type 2 diabetic patients, 42% were not treated with lipid lowering drugs, 74% of those treated did not reach the therapeutic goal. Half of the patients who reached LDL-C level <70 mg/dl remained at theoretical high risk because of low HDL-C and/or high serum triglycerides.

ENSURING LONGER LIFE STUDY. A NEW MODEL FOR AN INTEGRATED MANAGEMENT OF PATIENTS AT HIGH CARDIOVASCULAR RISK

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Background. The cardio-cerebrovascular diseases (CCV) are the most important causes of morbidity and mortality in Western countries. Although prevention strategies are well known and discussed, their implementation in medical practice is still unsatisfactory. This is of particular importance as it is known that the vast majority of events is potentially avoidable: in fact, over 80% of cases of myocardial infarction are attributable to a few well identified and modifiable risk factors.
Aim. The Ensuring Longer Life (ELL) study aims to acquire, in a standardized and controlled way, information of clinical interest in patients at high CCV risk; monitoring the long term appropriateness and adherence to the therapeutic regimen; integrate the new information with those already available in the Abruzzo Region, in order to improve health planning; providing a suitable model of Hospital-Country infrastructure, to support the process of "clinical governance".
Methods and Materials. The cornerstone of the project will be the platform "Quick opeNETica", which can provide fast and simple data collection by GPs, data sharing and transmission to a specialized coordinating center that will handle the analysis and interpretation of data. In fact, Quick allow the practical implementation of "Integrated Projects" between specialists and GPs. It ensure the possibility of: staging the patient; choose the most appropriate drug and dosage based on the grade of severity; report the type of prescription and justification for the monitored medications; controlling all the process indicators and outcomes; verify the achievement of the standards of appropriateness. 40 GPs throughout Chieti and Pescara ASLs participate in the project. They will record the information about 30 patients each (adults at high CCV risk in secondary prevention) in their medical records and perform data transmission to a dedicated server every four months for one year. Data will be analyzed and results will be available immediately after.

LDL-APHERESIS ABRUZZO NETWORK: AN OLD-NEW OPPORTUNITY FOR THE MANAGEMENT OF PATIENTS AT HIGH CARDIOVASCULAR RISK

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Background. Familial hypercholesterolemia (FH) represents a major risk factor for cardiovascular events. Patients are characterized by high levels of LDL-cholesterol (LDL-C) due to a deficit of the gene encoding the receptor for LDL-C, that explains their resistance to the (poly-)drug treatment, even when administered at high doses and/or in combination. High doses of statins may also have problems of tolerability, so that such patients are unlikely to reach recommended therapeutic targets. Since many years there is the possibility of treatment with LDL apheresis (LDL-A), but this method is still too little used in Italy compared to other European countries and, while considering costs, we must not overlook the benefits that may result from its larger diffusion all over the territory. AIM: To create a regional network consisting of a group of specialists in metabolic diseases and in apheresis, that could offer a new treatment opportunity for high cardiovascular risk patients refractory to pharmacological treatment.
Methods. We defined new regional guidelines to better identify which patient can really benefit from apheresys treatment: homozygous FH (HoFH) with LDL-C >500 mg/dL; double-heterozygous FH (He-HeFH) in primary prevention (LDL-C >300 mg/dL after 2 months of treatment with maximum doses of statins), heterozygous FH (He-FH) in primary prevention, if they are very-high-risk patients (>40% based on Italian risk calculator) non-responders (LDL-C >130) to therapy (3 months with the highest doses of statins), or not-tolerant (ALT, AST >3x UNL; CPK >4x) hyper-Lp(a) (>60 mg/dL); hyper-fibrinogenemia (>360 mg/dL); teens <18yo, if LDL-C >300 mg/dL (statins contraindicated).
Results. To-date we enrolled 4 new high-risk patients (M:F=3:1; mean age 57,2) refractory to medication (lack of efficacy, intolerance). We treated one blood volume per procedure with H.E.L.P. device every 10 days (7-21). They all show a good compliance (median duration of treatment = 11 months mean =15) and have a good efficacy (mean starting LDL-C =211 mg/dL, range 185-275; LDL-C at this time =128 mg/dL, range 122-145). LDL-A also improved statin tolerability as it has been associated with moderate dose of statin without significant AEs.
Conclusion. The integrated management of high-risk patients with severe hypercholesterolemia refractory to medication may offer valuable opportunity to reduce their cardiovascular risk.

HIGH-FAT DIET, VASCULAR LESIONS AND SYSTEMIC LEUKOCYTES RECRUITMENT: INSIGHTS FROM A PRECLINICAL ANIMAL MODEL

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Introduction. An increasing number of people in Western countries is subject to metabolic disorders linked by a systemic pro-inflammatory state, which includes vascular diseases, dyslipidemia and fatty liver disease.
The architecture of arteries, liver and white adipose tissue (WAT) is characterized by a close interaction between parenchymal and immune cells. Statins are inhibitors of cholesterol biosynthesis but they also exert a broad range of anti-inflammatory effects that inhibit leukocytes motility and adhesion to the vascular wall, reducing the amount of tissue-infiltrating leukocytes. The aim of this study was to evaluate systemic and tissue-specific inflammation in pigs fed a high-fat diet.

Materials and Methods. 21 pigs were kept for 16 weeks on a standard diet (SD, n=7) or a high-fat diet (HFD, n=14). Seven HFD-fed pigs received high-dose atorvastatin (80 mg/die) starting after 8 weeks of HFD, until the end of the experimental procedure. Balloon angioplasty was used to induce vascular injury in the left common carotid artery of pigs after 8 weeks of diet.
Results. The HFD exacerbated the development of vascular injury compared to the SD. HFD administration significantly raised the number of circulating leukocytes, particularly monocytes; showing a correlation between circulating white blood cells and the degree of vascular stenosis. HFD-fed pigs displayed adipocytes hypertrophy and T-lymphocytes infiltration in peri-adventitial and abdominal WAT. The HFD caused liver inflammation with increased infiltration of macrophages, T- and B-lymphocytes. Atorvastatin significantly reduced vascular injury, abolished WAT inflammation by reducing adipocyte area and the number of infiltrating T-lymphocytes. Moreover, atorvastatin decreased liver inflammation in HFD-fed pigs.
Conclusion. The HFD worsened the vascular injury, raised the amount of circulating leukocytes and exacerbated the inflammatory response in WAT and liver. Atorvastatin treatment significantly reduced vascular injury and markedly decreased the development of a systemic and tissue-specific inflammatory milieu as well as the accumulation of infiltrating leukocytes.

THE ROLE OF OSTEOPROTEGERIN, RANK-LIGAND, MATRIX-GLA PROTEIN AND C-TYPE NATRIURETIC PEPTIDE ON THE VASCULATURE OF NORMAL AND DIABETIC SUBJECTS

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Vascular calcification, a degenerative process considered in the past to be a passive procedure, has now been suggested as being related to the ossification process. Several factors known to be involved in the process of bone formation have been identified also on arterial walls. Among these the osteoprogeterin OPG/RANKL/RANK axis and the vitamin K dependent matrix GLA protein (MGP) have been reported to play an important role in the atherosclerotic process. Finally, C-type natriuretic peptide (CNP) plays a central role in the control of vasculature tone and in several clinical conditions characterized by endothelial dysfunction. This study aimed to investigate the relationships between OPG, RANKL, MGP and NT-pro CNP serum levels and carotid atherosclerosis. We studied 273 subjects, 178 normals and 95 with type 2 diabetes (DM2), (mean age: 56.6±7.5 yrs). In all subjects we measured OPG, RANKL, MGP and NT proCNP serum levels by ELISA methods. Intima media thickness and carotid plaque echogenicity were also assessed by ultrasonography in all subjects.MGP resulted significantly lower in DM2 patients than in normal subjects (7,39±2,2 nmol/l vs 12,9±6,4 nmol/l; p<0.01). In both normal subjects and DM2 patients MGP was inversely associated with carotid stenosis (r=-0,15; p <0,05 and r=-0,17; p<0,05). A significant correlation between MGP and IMT was found only in DM2 patients (r=-0,17; p<0,05). OPG resulted higher in DM2 patients than in normal subjects. NT-proCNP was lower in DM2 patients than in normals (p<0.05); moreover NT-proCNP was inversely associated with carotid stenosis in DM2 patients (p<0.05) but not in normal subjects. In conclusion, our findings seem to demonstrate that OPG, MGP and NT-proCNP play an important role in the carotid atherosclerotic process, so confirming the presence of links between mineral metabolism and cardiovascular diseases.

PLATELET AND ENDOTHELIAL ACTIVATION AND PROGRESSION OF COGNITIVE IMPAIRMENT IN VASCULAR DEMENTIA

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Multi-infarct dementia, also known as vascular dementia, is the second most common form of dementia after Alzheimer disease in older adults in westernized countries where it represents a relevant clinical and socioeconomic problem due to its deep impact on quality of life and prognosis. Vascular dementia is caused by different mechanisms all resulting in vascular lesions in the brain. In this regard, current concept on atherogenis identify in the proatherogenic activation of vascular endothelial cells and platelets a fundamental step in the onset and progression of atherosclerotic vascular damage. Starting from these evidence we aimed to evaluate the role of patherogenic vascular inflammation in the progression of cognitive impairment in patients with vascular dementia.
To address this topic we assessed circulating levels of soluble (s) CD40L and P-selectin, as indices of platelet activation, and of ICAM-1 and VCAM-1, as indices of endothelial activation in 78 patients (73.2±4.2 years) with newly diagnosized vascular dementia (NINDS-AIREN criteria) and in 56 healthy sex and age-matched control subjects (75.2±4.1 years). After baseline evaluations patients with vascular dementia were reevaluated every six months up to 2 years. At baseline, circulating biomarkers of endothelial (sICAM-1: 345.5±112.5 vs 188.2±65.0 ng/mL, p<0.0001; sVCAM-1: 634.2±132.3 vs 443.2±125.3 ng/mL, p<0.002) and platelet activation (sCD40L: 7.2±2.4 vs 2.9±0.9 ng/L, p<0.0001; sP-selectin: 67.3±22.2 vs 54.1±8.1 ng/mL, p<0.003) were higher in demented patients than in controls. At the end of follow-up period, a progression of cognitive impairment was observed in demented patients with a mean decrement of MMSE score of 3.2±1.0 (range 0.4-4.7).
Spearman non-parametric correlation found significant relationships between changes of MMSE score at the end follow-up and baseline circulating levels of sCD40L (r=0.452, p<0.002) and sICAM-1 (r=0.533, p<0.001). In addition, by multivariate regression analysis baseline sCD40L and sICAM-1 levels were found to be independent predictors of MMSE score decrement after adjustment for potential confounders, explaining about 10% and 12% of MMSE score changes, respectively. In conclusion, our data demnstrated the existance of a tight relationship between the degree of proatherogenic activation of vascular endothelial cells and platelets and the progression of cognitive impairment in patients with vascular dementia. These results, if confirmed in larger study population, could provide an useful tool to identify demented patients at high risk for disease progression and to evaluate the potential efficacy of intervention strategy aiming to least slow down the progression of dementia.

ROLE OF CD40/CD40L SYSTEM IN THE PATHOPHYSIOLOGY OF COGNITIVE IMPAIRMENT IN OLD HYPERTENSIVE PATIENTS WITH NON-DIPPER BLOOD PRESSURE PROFILE

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Hypertension is a risk factor for cognitive impairment and dement. The evidence of a tight relationship between blood pressure and cognitive impairment, which is already evident starting from high-normal blood pressure values, suggest the existence of a continuum of cognitive damage in hypertensive patients. According to this, a relationship between cognitive impairment and target organ damage has been described in hypertension.
The current study was designed to evaluate whether or not non-dipping blood pressure profile could be associated with impaired cognitive impairment in hypertensive patients. Furthermore, starting from previous evidence from our group of an involvement of CD40/CD40L in the pathophysiology of vascular damage in hypertensive patients(Desideri et al., Am J Hypertens 2007) and in cognitive impairment progression in Alzheimer disease (Desideri et al. Neurobiol Aging 2008), we also assessed the potential involvement of this system in the pathophysiology of cognitive impairment in non-dipper hypertensive patients. We studied 2 groups or 25 never-treated old hypertensive patients (age >75 years), without additional cardiovascular risk factors, clinically evident cerebrovascular disease or overt dementia, differentiated on the basis of a nocturnal decrease of BP either of>10% (dippers) or <10% (nondippers) of daytime values, and in 25 matched normotensives.
Cognitive function were assessed in all participants by MMSE, Trail Making Test A and B, Verbal Fluency Test. The result of these tests were logarithmically transformed and used to calculate a composite cognitive score.
Circulating levels of soluble CD40L and the lipid peroxidation product 8-iso-prostaglandin(PG)F2alfa were also assessed. Cognitive performance were found to be worse in hypertensive patients in comparison to normotensive subjects (-0.26±0.06 vs -0.13±0.06, $p<0.001$) expecially in those with blunted nocturnal blood pressure fall (non-dipper: -0.30±0.04, dipper: -0.23±0.05, $p<0.001$). Circulating levels of soluble CD40L (non-dipper: 4.7±1.5 ng/mL; dipper: 4.0+1.3 ng/mL; controls: 3.0±1.5 ng/mL) and 8-iso-PGF2alfa (non-dipper: 387±123 pg/L; dipper: 314±110 pg/L; controls: 271±53 pg/L) were significantly higher in non-dipper than in dipper hypertensive patients and controls ($p<0.01$). In the whole study population significant relationships between circulating soluble CD40L levels, cognitive score (r:0.369, $p<0.001$) and plasma levels of lipid peroxidation products (r:0.557, $p<0.0001$) were found. In a multivariate regression analysis sCD40L was found to be an independent determinant of cognitive impairment in the study population.
The results of our study suggest that non-dipping blood pressure profile is associated with cognitive dysfunction in hypertensive patients probably due to an overactivation ofCD40/CD40L system.

MOLECULAR STUDY OF GLUCOSE CONTROL AND INFLAMMATORY RESPONSE IN ANIMAL MODEL AND TYPE 2 DIABETES MELLITUS PATIENTS

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Introduction. Liver steatosis is a metabolic dysfunction associated with obesity, diabetes and hyperlipidemia. Utilizing proteomic analysis we observed that livers of diabetic IRKO mice (Insulin Receptor Knockout) expressed at least 44 proteins including HMGB1 and PRDX6. Interestingly, literature data and our preliminary results suggest that HMGB1 and PRDX6 may be deeply involved in the pathogenesis of type 2 diabetes (T2D). In this study, we hypothesized that HMGB1 is involved in the steatosis pathogenesis of T2D while glucose metabolism regulates the expression of PRDX-6 in diabetic subjects.
Materials and Methods. Utilizing ELISA methodology, we assessed the HMGB1 content of serum obtained from peripheral blood of 30 T2D patients (pts), 29 steatotic pts divided as follows: 17 with high level steatosis and 12 with low level of steatosis. The score of steatosis was determined using ultrasound and was graded by Needlemann method, (0=none, 1=mild, 2=moderate, 3=severe). Serum levels of IL-6 and TNF-α were measured in all pts. For evaluating the role of PRDX6 we bred IRKO+/- mice with PRDX6-/- mice obtaining 6 type of off springs: IR+/+/PRDX6+/+, IR+/+/PRDX6+/- IR+/+/PRDX6-/- IR+/-/PRDX6+/+, IR+/-/PRDX6+/-, IR+/-/PRDX6-/-.
Then, we analyzed serum levels of glucose and insulin by glucose tolerance test (GTT) in IR+/+/PRDX6+/+, IR+/+/PRDX6+/- IR+/+/PRDX6-/- IR+/-/PRDX6+/+, IR+/-/PRDX6+/-, IR+/-/PRDX6-/- mice. We analysed two mice groups for every genotype: young and elder. Insulin signalling alterations were studied by Real Time PCR and Western Blot analysis.
Results. We observed a modulation of HMGB1 secretion in T2D pts with or without hepatic steatosis. In D2T with low steatosis (score 0-1), HMGB1 levels was 4.7± 3.4ng/ml whereas pts with high steatosis (score 2-3) had 2.9±1.8 ng/ml serum levels of HMGB1. As we expected, pts with high steatosis had increased levels of TNF-α 9.7±11.2 pg/ml and IL-6 9.5±5.6 pg/ml; while in low steatosis conditions, serum concentration of TNF-α was 4.9±8.5 pg/ml and IL-6 concentration was 6.3±2.5 pg/ml. Pearson correlation coefficient showed a positive relation between HMGB1 and TNF-α ($\beta=0.33$, $p<0.05$).
Analysing partial correlation between HMGB1, TNF-α, IL-6 and/or steatosis score we obtained a positive correlation of HMGB1 with TNF-α ($\beta=0.44$, $p<0.001$) and HMGB1 with IL-6 ($\beta=0.29$, $p<0.005$). In the animal study we found that young IR+/+/PRDX6+/- and IR+/+/PRDX6-/- mice had an impaired glycemic control while elderly mice had normal glucose tolerance. The level of PRDX6 protein expression in young IR+/+/PRDX6+/- and IR+/-/PRDX6+/- mice was reduced in the liver tissue but was normal in the muscle. Elderly mice liver IR+/-/PRDX6+/+ and IR+/-/PRDX6+/- had an increased PRDX6 expression when compared with single or double knockout.
Our results showed also an hepatic reduction of PRDX6 linked with lower AKT-1 phosphorylation in Ser473. In insulin resistance models (IR+/-), we noted an increase level PEPCK protein expression when AKT-1 phosphorylation in Ser473 was reduced. As in liver as in muscle tissue, the lack of both genes induced

insulin resistance including reduction of AKT-1 phosphorylation in Ser473.
Conclusions. HMGB1 has a function in inflammation state activated in Diabetes Mellitus Type 2 but not in steatosis. In fact we observed, a linear positive correlation between HMGB1, TNF-α and IL-6 in pts with steatosis score 0-1 but not in pts with high steatosis levels (score 2-3).
PRDX6 seems to be a new insulin substrate since a reduced expression of PRDX6 leads to an impaired glucose tolerance state and its expression can increase hepatic insulin resistance by affecting the PI3K-AKT1 pathway.

CRP IN THE ITALIAN POPULATION: THE CHECK STUDY

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Few data are available on the distribution of C-reactive protein (hsCRP), a major inflammatory biomarker, in the Italian population. The aim of this analysis was to determine distribution, frequency of occurrence of elevated hsCRP and possible relationship with other cardiovascular risk factors in a representative cohort of Italian population. CHECK is a randomized Italian epidemiological study conducted in 5846 subjects, between 40 and 79 years.
HsCRP was assessed on frozen plasma samples from 2370 randomly selected participants to the study. HsCRP distribution was non-normal; median (IQR) was 1.30 (0.57-2.65), higher in women [1.42 (0.58-2.86) vs 1.28 (0.58-2.51); NS] and increasing with age [from 0.92 (0.40-2.08) in 40-49 years to 1.92 (0.99-3.40) in 70-79 years; p<.001]. 35.6% (38.2% W vs 33.1% M) of the study population had elevated hsCRP values (>=2 mg/L).
Stepwise regression analysis between circulating CRP (log-transformed dependent variable) and several covariates showed significant positive associations with number of metabolic syndrome determinants, fibrinogen, BMI, apoB, age, triglycerides, and significant negative associations with total cholesterol, physical activity, creatinine clearance (MDRD formula), and educational level. HsCRP levels progressively increased among global CV risk classes (assessed with CUORE algorithm), from 1.01 (0.45-2.21) in very-low-risk subjects (<5%) to 2.03 (0.97-3.42) in high-risk subjects (>=20%) (p<.001).
Median levels of this marker were higher in treated subjects, either with dietary intervention or with pharmacological therapies for hypertension, diabetes, and hypercholesterolemia, with significant differences, except for hypolipidemic treatment. In summary, CRP plasma concentration was linearly associated with several conventional CV risk factors. This evidence is consistent with other observations, but not for BMI.
The increased hsCRP levels across CV risk classes perhaps reflect the correlation of this marker with some of the contributors to the score determination.
The CHECK study was supported in part by an unconditioned educational grant from AstraZeneca SpA.

CARDIOVASCULAR RISK FACTORS: THE GAP BETWEEN ESC RECOMMENDATION AND PRACTICE IN THE CHECK STUDY

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Although international guidelines underline the benefits of managing major cardiovascular risk factors, many patients who should be receiving treatment are not. The aim of this analysis was to describe a sample of Italian population in respect of ESC 07 goals for blood pressure and cholesterol. CHECK was a large randomized epidemiological study, conducted by Italian general practitioners on 5846 subjects, (40-79 years old). According to ESC guidelines, a subsample of the study cohort was classified in two categories of CV risk, in order to define their specific therapeutic targets for blood pressure (BP) and lipids (TC and LDL-c). We considered only subjects without antihypertensive and hypolipidemic treatments, which may have influenced basal pressure and lipid levels. Of 3358 subjects without specific treatments (50.0% men; mean age 54.5 years), 17.7% (25.1% M and 10.4% W) were at high risk (HR). In this subgroup, 85.2%, 93.4%, and 91.8%, were not at target for BP, CT, and LDL-c, respectively; in low-risk (LR) subjects, percentages were 16.7%, 65.3%, and 61.8%. Low HDL-cholesterol and high triglycerides, regarded as markers of increased cardiovascular risk by ESC guidelines, were present in 11.5% and 23.3% of all subjects. According to ESC recommendations, among subjects not a target for BP 24.4% would require life style counselling, 10.9% a pharmacological treatment, and in 64.4% the type of intervention should be chosen after clinical evaluation of cardiovascular status. Among subjects not at target for lipid levels, respective proportions were 73.9%, 10.2%, and 12.1%. In this primary care population of non-treated subjects, almost one fifth was in HR class. Overall, considering specific targets (BP, TC and LDL-c) for the two risk classes, only 21% of subjects were under recommended levels. Among not-at-target subjects, at least one out eight should be addressed to antihypertensive or hypolipemic therapies. The CHECK study was supported in part by an unconditioned educational grant from AstraZeneca SpA.

RESEQUENCING OF LDL RECEPTOR GENE (LDLR) IN A COHORT OF ITALIAN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA (FCHL): THE APPLICATION OF THE MUTATION ACCUMULATION STRATEGY TO A COMPLEX LIPID DISORDER

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Background. Familial combined hyperlipidemia (FCHL), characterized by elevation of both low density lipoprotein cholesterol (LDL-C) and total triglycerides (TG) within families, is a com-

plex genetic disorder, whose molecular bases are poorly defined. A promising new strategy to understand common complex genetic traits is called "mutation accumulation approach", which aims to detect the enrichment of rare, deleterious DNA variants in affected individuals. Due to the combined hyperlipemic phenotype, it is plausible to postulate that FCHL may be caused by accumulation of multiple DNA variants in genes regulating both LDL and TG metabolism, namely the LDL receptor (LDLR) and the lipoprotein lipase (LPL) coding genes. This is supported by a previous observation ofa 20% frequency of mutations in LDLR in a Spanish cohort of FCHL.
Aim. Within a project of re-sequencing of the genes involved in lipid metabolism in FCHL, we preliminary report the prevalence of non-synonimous (NS) sequence variations in the LDLR in an Italian cohort of 84 unrelated FCHL patients.
Methods and Results. In FCHL patients showing elevation of LDL-C (IIA and IIB phenotypes) (n=51; LDL-C 203.9±32.0 mg/dl)) or isolated hyperapoB (n=32; apoB levels 162.4±19.4 mg/dl), LDLR was resequenced. Coding regions and intron-exon boundaries of LDLR were directly sequenced in 5' and 3' directions in ABI 3730 or 310 DNA Analyzer.We found n=4 NS variants; all were already known and two (P664L,T41M) have been associated to familial hypercholesterolemia (FH); among the others, G-2R can predicted as damaging, while the A370T as benign. The overall the prevalence of LDLR mutations in this FCHL cohort was 4.8%.
Conclusions. Mutations in LDLR can be found in FCHL, probably due to the phenotypic overlap of FH and FCHL. However, their prevalence is lower than previously reported, confirming that LDLR gives a limited contribution to the lipid phenotype in this syndrome.

LEFT VENTRICULAR HYPERTROPHY AND ARTERIAL STIFFNESS ARE ASSOCIATED IN ESSENTIAL HYPERTENSION PATIENTS

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Background. It is now well known the importance of target organ damage in the assessment of cardiovascular risk in hypertensive patients. Aim of our study is to describe whether there is an association between concentric left ventricular hypertrophy and arterial stiffness, in a large population of essential hypertension patients.
Methods. By standard trans-thoracic echocardiography we estimate anatomical (left ventricular mass indexed by body surface area [LVMI] and relative wall thickness [RWT]) and functional (ejection fraction [EF], diastolic function [E/A] and deceleration time [dec T]) parameters on 827 treated hypertensive patients. Furthermore, we measured blood pressure (BP, sphygmomanometer) and carotid-femoral pulse wave velocity (PWV, Complior).
Results. Patients were 53±14 years old and 50% were male. Mean systolic BP and diastolic BP were 142.3±18.6/86.7±10.6 mmHg. Mean LVMI was 111.36±32.7 g/m^2, RWT 0.41±0.07, EF 63±4%, E/A 1.05±0.33, dec T 211±47 msec, while PWV was 10.7 m/Sec. PWV was significantly correlated with LVMI (r=0.214, p<0.001), E/A (r=-0.25, p<0.001) and dec T (r=0.146, p<0.001). According to ESH-ESC guidelines in 336 (43%) patients we found a normal left ventricular geometry, while in 163 (21%) we found concentric remodelling, in 173 (22%) concentric Hypertrophy and in 109 (14%) eccentric hypertrophy. PWV was significantly different between the 4 subgroups (p=0.001), and patients with concentric and eccentric hypertrophy also showed higher PWV values (p≤0.001 for both).
Conclusion. In EH patients arterial stiffness is associated with the degree of cardiac damage. This finding may be helpful in understanding interactions between vascular and cardiac dysfunction in the continuum of cardiovascular disease.

RENAL AND VASCULAR DAMAGE ARE CONNECTED IN AIDS PATIENTS

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Background. Although combination antiretroviral therapy (ART) has improved life expectancy of AIDS patients, it has been observed a higher cardiovascular mortality and a greater prevalence of target organ damage even in absence of arterial hypertension.
Aim. Aim of this study was to describe the influence of renal damage (RD) and ART on arterial function and structure. Methods: We studied 4 groups of normotensive, normocholesterolemic, euglycemic patients; one of HIV+ on ART with RD (A; n=25; age 50.2±10.4 years; means±SD), one of HIV+ on ART without RD (B; n=25; 49.4±6.2 years), one of HIV+ not on ART and without RD (C; n=13; 40±8.3 years) and one of healthy controls (D; n=25; 50±6.8 years). Renal damage was defined by the presence of microalbuminuria and/or estimated glomerular filtration rate (MDRD) <60 ml/min. Arterial stiffness was measured by carotid-femoral Pulse Wave Velocity (PWV,Complior), central BP by applanation tonometry (Sphygmocor) and carotid artery intima-media thickness (IMT) by semi-automatic echotracking (WTS).
Results. Group A showed higher aortic SBP and PP than other groups and their aortic SBP was not significantly different from systemic one. PWV was higher in both therapy groups compared to others. IMT didn't show any difference among groups.
Conclusion. No alteration in arterial structure and function is present in normotensive AIDS patients, while arterial damage is present in AIDS patients also affected by renal damage and this could be due to an increase in central BP, which may account, at least in part, for their increased cardiovascular risk.

LONG-TERM EFFECTS OF SUBACUTE HDL INFUSION IN A RABBIT MODEL OF ATHEROSCLEROSIS

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HDL therapy reduces atherosclerosis in animal models, and small clinical studies support the conclusion that synthetic HDL

may represent a treatment option for atherosclerosis regression in humans. However, the efficacy of HDL therapy has been evaluated only shortly after treatment, therefore long-term effects of HDL therapy have not been yet elucidated. The aim of the present study was to assess long-term effects of synthetic HDL administration on plaque size and markers of plaque vulnerability in a rabbit model of atherosclerosis. Advanced aortic lesions were induced in 24 New Zealand White rabbits by double balloon injury and 0.2% cholesterol diet. Plaque size was assessed by MRI at the end of atherosclerosis induction. Animals were randomized in two groups and treated with placebo or synthetic HDL constituted by apoA-IMilano and phospholipids (ETC-216). Rabbits received 2 infusions, 4 days apart. After the last dose, another MRI analysis was performed, and then rabbits were maintained at 0.1% cholesterol diet for the following 6 months. After plaque size measurement by MRI, rabbits were sacrificed and aortas were processed for subsequent evaluations. ETC-216 treatment induced a significant plaque regression ($p<0.05$ vs. pre-treatment) that was maintained at 6 months after treatment, whereas the placebo showed no significant effect. Plaque regression after 6 months from ETC-216 treatment was also associated with a reduced expression of MCP-1 and a decrease in gelatinolytic activity. Interestingly, immunostaining of plaques with a specific antibody directed against human apoA-IMilano was still positive at 6 months after infusion. In conclusion, plaque regression observed after short-term ETC-216 administration in rabbits was maintained for at least 6 months after treatment and was associated with a significant reduction in potential makers of plaque vulnerability. The prolonged permanence in the plaque of immunoreactive apoA-IMilano, may account for the long-lasting effect of HDL therapy on plaque size/stability.

PREVALENCE AND PROGNOSTIC VALUE OF DIABETES MELLITUS IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION FOR ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION

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Background and Aims. The Percutaneous Coronary Intervention (PCI) registry of Cardiology Division of Trieste, investigates the course of diabetic and nondiabetic patients with ST-segment elevation myocardial infarction (STEMI). The aim of this study was to assess the prevalence of Diabetes Mellitus (DM) and its influence on the effectiveness of mechanical reperfusion and outcome in patients with STEMI who underwent PCI.
Materials and Methods: 794 patients with STEMI undergone to PCI were enrolled from PCI registry of Trieste (anamnestic, clinical, angiographic, echocardiographic and therapeutic data) between December 1,2003, and December 31, 2009. One month and six years follow up is being conducted to quantify patients' postdischarge outcomes.
Results. Of 794 patients, 192 (24%) had DM (anamnestic or unknown). Diabetic subjects had higher mean age ($p\leq0.0001$) with prevalence of male gender ($p\leq0.004$), more prevalence of arterial hypertension ($p\leq0.0001$), peripheral arteriopathy ($p\leq0.007$), higher heart rate ($p\leq0.011$), Killip class ($p\leq0.001$), triglycerides ($p\leq0.004$) and lower HDL cholesterol ($p\leq0.027$). Both groups had the same mechanical reperfusive treatment, but diabetic one had worst outcome after PCI with medium-severe ventricular disfunction ($p\leq0.041$) and congestive heart failure ($p\leq0.0001$). Both groups also had same pharmacological therapy. According to Kaplan Mayer survival analysis, diabetics had higher mortality at 30 days and 6 years following STEMI, increasing in the course of time ($p\leq0.001$).
Conclusions. Despite optimum reperfusive and pharmacological treatment for STEMI, diabetes confers a significant adverse prognosis, which highlights the importance of aggressive strategies to manage cardiovascular risk factors before progression of cardiovascular disease.

ENDOMORPHIN-1 PREVENTS LIPID ACCUMULATION VIA CD36 DOWN-REGULATION AND MODULATES CYTOKINES RELEASE FROM HUMAN LIPID-LADEN MACROPHAGES

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Aim. CD36 is a scavenger receptor known to play a critical role in the development of atherosclerosis by mediating the uptake of oxidized low-density lipoproteins (oxLDL) by macrophages, thus leading to foam cell formation. It is now generally recognized that the immune system has a pivotal role in the pathogenesis of atherosclerosis, whose progression is determined by ongoing inflammatory reactions.
Recently, several studies pointed out that opioid peptides exert anti-inflammatory activities. In this study, we investigated the effect of endomorphin-1 (EM-1) on lipid accumulation as well as on modulation of CD36 expression and cytokines release from human foam cells.
Methods and Results. Foam cells were obtained both by human THP-1 monocytic cell line and by isolated human peripheral blood macrophages.
Fluorescence and Confocal Microscopy was used to measure the intracellular lipid-droplets content. Flow Cytometry and molecular biology methods were used to evaluate of CD36 scavenger receptor. The levels of inflammatory cytokines was also evaluated by means of ELISA technique.
The results showed that EM-1-treated macrophages contained less Nile Red-stained lipid droplets. Furthermore, EM-1significantly decreased the expression of CD36 receptor and tumor necrosis factor-α (TNF-α) release, while increased the release of interferon-γ (IFN-γ).
Naloxone, a selective μ-opioid receptor antagonist, impaired the abilities of EM-1-treated foam cells either to accumulate modified lipids by down-regulating CD36 receptor expression or to act as an anti-inflammatory agent in terms of cytokines production.
Conclusions. We have demonstrated, for the first time, an unprecedented ability of EM-1 to act as a novel modulator for macrophage-to-foam cell transformation, and to modulate the production of inflammatory cytokines by foam cells. These results advocate for possible novel endomorphin-based anti-atherosclerotic approaches for the prevention and treatment of atherosclerosis.

BLOOD PRESSURE LOWERING EFFECT OF LACTOTRIPEPTIDES ASSUMED AS FUNCTIONAL FOODS: A META-ANALYSIS OF CURRENT AVAILABLE CLINICAL TRIALS

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Background. The oral assumption of lactotripeptides Valine-Proline-Proline (VPP) and Isoleucine-Proline-Proline (IPP) as nutraceuticals or functional foods is supposed to improve blood pressure control by ACE-inhibition. However data derived from clinical trials have reached conflicting conclusions. Our aim was to perform a meta-analysis of placebo-controlled clinical trials evaluating the antihypertensive effect of lactotripeptides assumed as nutraceuticals or functional foods.
Design. Trials identified using a defined search strategy in PubMed were included in the meta-analysis, and their pooled effect was estimated with a random effects model, weighting for the inverse of the variance. Heterogeneity, publication bias, subgroup, and meta-regression analyses were performed.
Results. 18 trials have been identified whose clinical data have been clearly reported. Pooled effect of peptides was a reduction of -3.73 mmHg (95% CI: -6.70, -1.76) for systolic blood pressure (SBP), and 1.97 mmHg (95% CI: -3.85, -0.64) for diastolic blood pressure (DBP). The effect was more evident in Asian patients [SBP: -6.93 mmHg (95% CI: -10.95, -2.94); DBP=-3.98 mmHg (95% CI: -5.38, -2.44)] than in Caucasian ones [SBP = -1.17 mmHg (95% CI: -2.82, 0.72); DBP= -0.52 mmHg (95% CI: -1.39, 0.13)], and apparently not related to age, baseline blood pressure values, dose of lactotripeptides assumed or length of the treatment.
Conclusions. VPP and IPP lactotripeptides assumed as functional foods may significantly reduce SBP particularly in Asian subjects. The relevance of this findings in other ethnicities or associated to different dietary pattern should to be further investigated.

EFFECT OF LACTOTRIPEPTIDES ASSUMED AS FUNCTIONAL FOOD ON OFFICE AND 24-HOUR BLOOD PRESSURE, STRESS-INDUCED BLOOD PRESSURE INCREASE, PULSE WAVE VELOCITY AND CARDIAC OUTPUT RELATED PARAMETERS: A RANDOMIZED, DOUBLE-BLIND, CLINICAL TRIAL

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Background. Contrasting data partially support a certain antihypertensive efficacy of lactotripeptides derived from enzymatic treatment of casein hydrolysate. Our aim is to evaluate this effect on a large number of haemodynamic parameters.
Methods. In the context of a double-blind, RCT, carried out on 52 patients affected by high-normal blood pressure or first degree hypertension, we evaluated the effect of 6 weeks of treatment with lactotirepeptides (LTP) IPP/VPP 3 mg/day assumed as functional food on the following parameters: office systolic, diastolic, pulse and mean pressure, 24-hour ambulatory systolic, diastolic, and pulse pressure, systolic, diastolic, pulse and mean stress-induced blood pressure increase, cardiac output, cardiac index, systemic vascular resistance, systemic vascular resistance index, stroke volume, stroke volume index, thoracic fluid content, acceleration index, left cardiac work index, pre-ejection period, left ventricular ejection time, velocity index, systolic time ratio.
Results. In the LTP treated subjects, we observed a significant reduction in office systolic blood pressure (-5±8 mmHg, p= 0.013) and to a significant improvement of pulse wave velocity (-0.66±0.81 m/s, p= 0.001) (instrumental biomarker of vascular rigidity).
No effect of both treatment has been observed as it regards 24 hour ABPM parameters and blood pressure reaction to stress.
LTP, but not placebo, are associated with a mild but significant change in Stroke volume and Stroke volume index (markers of cardiac flow) Acceleration index and Velocity index (markers of cardiac contractility).
No effect has been observed on parameters related to fluid dynamics or vascular resistance.
Conclusion. LTP assumed as functional food positively influence office systolic blood pressure, pulse-wave velocity, stroke volume, stroke volume index, acceleration index and velocity index in patients with normal high blood pressure or first degree hypertension

EFFECT OF A NUTRACEUTICAL CONTAINING ORTOSIPHON STAMINEUS LEAF EXTRACT ON BLOOD PRESSURE AND METABOLIC SYNDROME COMPONENTS IN HYPERTENSIVE DYSLIPIDAEMIC PATIENTS TREATED WITH CALCIUM-CHANNEL BLOCKERS OR ANGIOTENSIN CONVERTING ENZYME INHIBITORS

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Background. Different commercially available nutraceuticals are able to significantly reduce cholesterolemia, but there are less efficacious products that modulates blood pressure. Our aim was to comparatively evaluate the cardiovascular risk modulating effect of an Ortosiphon containing combined nutraceutical versus hydrochlorothiazide (HCTZ) when added to an stabilized antihypertensive therapy.
Design. Single-blind, centre-randomized clinical trial. Methods: This is a single-blind, centre-randomized clinical trial. Heighty patients in monotherapy with ACE-inhibitors or Calcium-Channel Blockers (CCBs) have been randomized to treatment with a combined nutraceuticals containing Ortosiphon stamineus 100 mg associated to berberine 500 mg and monakolin 3 mg, or to HCTZ 12.5 mg associated with intensification of life-style improvement for 8 weeks.
Results. Both treatments significantly reduced SBP, DBP and PP when compared to the baseline values, but HCTZ was more efficacious than Ortosiphon in reducing SBP (-9.50±6.66 mmHg

vs. 4.72±1.83 mmHg; p<0.001) and DBP (-5.55±4.14 mmHg vs. 2.37±2.32 mmHg; p<0.001). The lipid parameters significantly improved more in the combined nutraceutical treated group than in the HCTZ treated one (p<0.001 for all variables). The cardiovascular disease estimated risk improved more in the nutraceutical treated group than in the other patients (-7.39±5.92% vs. -3.36±9.79%; p=0.029).
At the baseline, 62.5% of subjects then treated with HCTZ and 47.5% of those then treated with the nutraceutical had 3 or more uncontrolled components of the metabolic syndrome (p=0.261). At the end of the treatment period, the HCTZ treated patients affected by metabolic syndrome decreased to 27.5% and 15.0%, respectively.
Conclusion. a combined nutraceutical containing Ortosiphon stamineus exert a significant reduction of systolic, diastolic and pulse pressure reduction in hypertensive dyslipidaemic subjects just treated with ACE-Is or CCB, even if less relevant than HCTZ. However the nutraceutical based approach had a more significant impact on a large number of risk factors and on the metabolic syndrome control.

ANTIHYPERCHOLESTEROLEMIC ACTIVITY OF A BRAND COMBINED NUTRACEUTICAL IN GENERAL PRACTITIONER CLINICAL PRACTICE

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Background. Large doses of monakolins are associated to an improved prognosis of patients affected by CAD. However, for safety reason, in Italy it has prescribed at a maximal dosage of 3 mg/day. This has pushed industries to develop combined nutraceuticals in order to improve this treatment efficacy in safe conditions.
Methods. This is an open unblinded study carried out in the context of a general practitioner clinical practice. A brand combined nutraceutical containing monakolins, policosanols and phytosterols (Liposcudil (R)) has been administrated during a month to 53 patients consecutively recruited by general physicians of the Emilia-Romagna region. The standard parameters of efficacy and safety have been monitored.
Results. No patient withdrawn from the study because of tolerability problems. Total cholesterol decreased by 34±30 mg/dL (p<0.001), LDL-C by 36±30 mg/dL (p<0.001) and non-HDL-C by 36±31 mg/dL (p<0.001). LDL-C, as main outcome of the study, was percentually reduced by a 20±16% (p<0.001). Non significant changes have been observed as it regards fasting plasma glycemia, other lipid parameters and safety parameters.
Conclusion. Liposcudil, a combined nutraceutical, exert a significant antihypercholesterolemic activity and is well-tolerated in the setting of general practitioner clinical practice.

EVALUATION OF THE SHORT TERM EFFICACY AND TOLERABILITY OF A COMBINED NUTRACEUTICAL WITH LIPID LOWERING PROPERTIES: A RANDOMIZED CLINICAL TRIAL

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Background. A large amount of literature suggests that numerous natural products could have interesting preventive activities in the management of human hyperlipidaemia. For statin-like dosage of monakolins we have also evidence of a reduction in morbidity and mortality in high risk patients. However, being these molecules analogues of statins, they also have the same safety profile and thus potentially relevant side effects.
Methods. This is a double-blind, placebo-controlled clinical trial on a combined nutraceutical non containing statin-like substances in 40 patients affected by primary polygenic hypercholesterolemia. After 4 weeks of ATP III life-style improvement, patients were randomized to assume a combined nutraceutical or placebo 1 pill/day for 8 weeks. The tested nutraceutical contained Octacosanols, Tocotrienols and Polymethoxylated flavones, (offered by Ca.Di.Group Srl, Rome, I).
Results. When comparing the combined nutraceutical effect with the one of placebo, we observed that the combined nutraceutical assumption was associated with a significantly higher decrease in Total Cholesterol (TC) (-17±2%, p<0.001), LDL-Cholesterol (LDL-C) (-22±3%, p<0.001), Triglycerides (TG) (-20±9%, p<0.001) and non HDL-Cholesterol (HDL-C) (-21±2%, p<0.001) than the control group. Non significant changes have been observed as it regards fasting plasma glycemia, other lipid parameters and safety parameters.
Conclusion. The tested combined nutraceutical is well tolerated and efficacious in reducing plasma lipid levels in subjects affected by primary polygenic hypercholesterolemia.

SOCIO-DEMOGRAPHIC AND HEALTH-RELATED FACTORS ASSOCIATED WITH LEISURE TIME PHYSICAL ACTIVITY LEVEL IN AN ADULT POPULATION SAMPLE: DATA FROM THE MASSALOMBARDA PROJECT

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Background. Leisure activity is associated to better outcomes in term of cardiovascular disease and perceived quality of life. Our aim was to estimate the adherence to the recommendation of daily leisure-time physical activity (PA) and to assess its association with socio-demographic factors and some health-related parameters in an Italian population sample.
Subjects and Methods. Our study involves 1 470 subjects (M: 678, W: 792; mean age: 56.9±16.8 years) visited during the 1st

survery of the Massalombarda Project. PA was assessed by using the Minnesota Leisure Time Physical Activity Questionnaire and classified as low (<450 METs.min.wk-1), moderate (450-750 METs.min.wk-1) and high (>750 MET.min.wk-1).
Results. A total of 55.5% of the study population was classified as either moderately or highly physically active. The highest proportion of subjects not adhering to the PA recommendation (60%) was found in the obese group. After adjustment for all studied correlates. The students, subjects living alone, with a BMI below 30 kg/m^2, and those rating their health from satisfactory to very good, had increased odds of being in the high PA category. Repeating adjusted analysis by gender, education and kind of cohabitants were significantly associated with the PA level in men, while BMI and marital status in women.
Conclusion. BMI, but also sociodemographic factors, are strong determinant of adherence to PA recommendation in large population sample.

THE IMPACT OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE ON ENDOTHELIAL FUNCTION: STUDY ON A GENETICALLY MODIFIED MODEL

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Familial LCAT deficiency (FLD) and Fish eye disease (FED) are two rare diseases due to about 60 different mutations in LCAT gene, with an autosomal recessive transmission. FLD and FED patients are characterized by a severe hypoalphalipoproteinemia. Since an altered vascular contractility and endothelial dysfunction are predisposing factors to cardiovascular diseases and this pathological sight is often associated to hypoalphalipoproteinemia, we investigated the vascular function in an animal model of LCAT deficiency. The study was performed on five wild-type mice as controls, five heterozygous LCAT knock-out mice and five homozygous LCAT knock-out mice. At the age of six months all mice were sacrificed, the toracic aorta was removed from each mouse and then the vessels were cleaned of fat and connective tissue, cut into 3 mm rings and suspended in organ baths containing Krebs' solution.
The rings were connected to isometric tension transducers coupled with a digital recording system able to record the entity of vascular contraction or dilatation. Increasing doses of different stimuli such as norepinephrine, acetylcholine, L-arginine methyl ester (L-NAME) and sodium nitroprusside were added to the bath, to obtain cumulative concentration-response curves. We observed that the rings of toracic aortas from homozygous and heterozygous LCAT knock-out mice were less responsive ($p<0.01$) to the contractile stimulus norepinephrine and to the vasodilating stimulus acetylcholine than those from wild-type mice. No differences were observed among the three groups in their response to sodium nitroprusside, whereas homozygous and heterozygous LCAT knock-out mice showed a lower contraction following L-NAME stimulus vs wild-type mice. In summary LCAT deficiency, in a mouse model, is associated with an altered vascular contractility and endothelial dysfunction. Studies on the expression of genes affecting the vascular tone are ongoing in the three groups, to elucidate the mechanisms behind the observed results.

IN RENAL TRANSPLANT RECIPIENTS BONE MARROW-DERIVED PROGENITOR CELLS ARE RELATED TO ENDOTHELIAL RESPONSE TO HYPEREMIA AND TO PARATHYROID HORMONE LEVELS

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Introduction. Patients undergoing renal transplantation are at high risk for vascular mortality, and endothelial dysfunction, a systemic disorder associated with cardiovascular events, may contribute to modulate cardiovascular complications in these patients.
Methods and Results. We investigated in 120 renal transplant recipients the relationship between bone marrow-derived progenitor cells (CPCs and EPCs) and both endothelial response to hyperemia, evaluated through digital pulse amplitude tonometry (PAT), and clinical, biohumoral and genetic parameters. We observed significantly lower RHI values according to the presence of three or more risk factors ($p=0.04$). EPCs were significantly correlated with RHI ($p=0.04$), and PTH ($p=0.007$). Among biohumoral parameters PTH showed a tendency to increase from the highest to the lowest tertile of RHI. In patients who underwent dialysis for more than five years, lower RHI values and EPCs number, and higher PTH concentrations in comparison to those observed in patients with less than one year dialysis time were observed. As concerns eNOS gene polymorphisms a trend to lower, even if not significantly, RHI value in subjects 4a/4a homozygotes was found.
Conclusions. The present study provides evidence for the relationship between progenitor cells and endothelial function detected by non invasive peripheral arterial tonometry and PTH, a new independent cardiovascular risk factor.

CARDIOVASCULAR RISK STRATIFICATION: THE ROLE OF CAROTI ULTRASONOGRAPHY

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Introduction. The prevention of atherosclerosis is based on the predictive power of risk factors such as age, sex, total cholesterol, HDL-cholesterol, current smoking, hypertension and diabetes mellitus. The predictive power of traditional risk factors is however low in the great majority of population classified as having an intermediate cardiovascular risk. In these patients, non invasive techniques, for instance B-mode ultrasound imaging combined with a Doppler assessment of flow, may identify the presence of atherosclerotic vascular lesions that may suggest a more aggressive approach.
Material and Methods. In a group of 258 subjects (133 women and 125 men) free of cardiovascular disease we evaluated: 1) the probability of having a cardiovascular event in the next 10 years

using the algorithm proposed by the Istituto Superiore di Sanità (ISS) for the italian population and by the most common used algorithm of Framingham. 2) the prevalence of clinically relevant carotid artery disease by ultrasonography. Carotid intima-media thickness >=1 mm or any discernible carotid plaque was considered indicative of carotid artery disease.
Results. Only 8% of the subjects had a risk >20% after calculation with the ISS algorithm, as compared with 22% with the Framingham algorithm. A total of 163 subjects (63%) had a carotid intima-media thickness <1 mm, 87 (34%) had a carotid intima-media thickness >=1 mm or carotid plaque causing a stenosis <50% and 8 (3%) had carotid plaques causing a stenosis >=50%. Of the subjects with carotid intima-media thickness >=1 mm or carotid stenosis <50%, those classified in the low-moderate risk group (10 year risk <10%) were 86% according the ISS and 67% according to the Framingham algorithm. Of the subjects with carotid stenosis >=50%, those classified in the low-moderate risk group were 63%, according to the ISS and 38% according to the Framingham algorithm.
Conclusion. Early detection of atherosclerosis with non invasive techniques improves the risk assessment beyond that provided by traditional risk factors. Ultrasonography, of carotid arteries might then be suggested in a comprehensive strategy of prevention of cardiovascular disease, in particular in those subjects classified at low-moderate risk by current algorithms.

SCREENING OF EXTRACRANIAL CAROTID ARTERY DISEASE IN PATIENTS WITH SYNCOPE, DIZZINESS, VERTIGO OR TINNITUS

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Introduction. The burden of patients suffering from syncope, dizziness, vertigo or tinnitus referred for ultrasound study of carotid arteries is high. Material and Methods: We conducted a study on 428 patients (178 males and 250 females) of whom 221 were referred for syncope, 164 for vertigo, 22 for tinnitus and 21 for dizziness. All the patients underwent B-mode ultrasound imaging study combined with a Doppler assessment of flow of carotid arteries. Cardiovascular risk was estimated according to the National Cholesterol Education Program ATP III.
Results. In 316 (74%) patients, carotid arteries did not have clinically relevant atherosclerotic plaques, in 62 (14%) patients we found a stenosis of 30-49%, in 37 (9%) a stenosis of 50-69% and in 13 (3%) a stenosis of 70% or higher. Prevalence of artery stenosis (≥50%) that could be considered for medical or surgical treatment was observed only in patients older than 65 years and was greatest in those with coronary heart disease or equivalent risk. Carotid stenosis Low-risk n 65 High-risk n 246 Coronary heart disease or equivalent risk n 117 n 37 n 28 n 71 n 175 n 29 n 88 <65 years ≥65 years <65 years ≥65 years <65 years ≥65 years 0-29% 100% 86% 99% 68% 76% 50% 30-49% 0% 7% 1% 21% 24% 18% 50-69% 0% 7% 0% 8% 0% 24% ≥70% 0% 0% 0% 3% 0% 8%
Conclusion. Carotid Doppler ultrasound is not recommended for patients under 65 years of age complaining of syncope, vertigo, dizziness or tinnitus. In older persons, ultrasound carotid study may be advisable, provided that the patients are at high cardiovascular risk or with coronary heart disease or coronary heart disease risk equivalents. In these patients, the probability to find carotid stenosis ≥50%, is relatively high (18%). Algorithm of the National Cholesterol Education Program ATP III may be useful in selecting asymptomatic persons with high probability of having high-grade carotid artery disease.

TOYOTISM PRINCIPLES AND APPLICATIONS IN AMELIORATING THE REGIONAL NETWORK FOR INHERITED LIPID DISORDERS PERCEPTION AMONG INTERNAL CUSTOMERS

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It is a general trend of today's hospital management to shift towards toyotism id est towards managerial modeling of clinical and organizational pathways based on Toyota derived principles. KAI ZEN which is the key concept of toyotism means to de construct a built setting of care (KAI) in order to construct a new better establishment (ZEN). This transformation support a continuous flow of add-value all over along the step. We assessed toyotism strategic implication in order to ameliorate the network perception among Federico II University Hospital Customers. Data set and formal and informal communication strategies were analyzed and revised together with coordination center participants. Informational strategies were classified by add-value incrementing and by their contribution to tacit knowledge

1. Clinical specific report, ADD VALUE +++ TACIT KNOWLEWDGE ++
2. Intra-net short communication, ADD VALUE ++ + TACIT KNOWLEWDGE ++
3. Work shop, ADD VALUE + + TACIT KNOWLEWDGE +++
4. Managerial report to chief executive data report. ADD VALUE + TACIT KNOWLEWDGE +

We reported that formal communications, albeit well constructed have a poor contribution to the network care perception among customers. It is crucial to change the strategy of diffusion of activity based data in order to gain a more complete and reliable add-value. Customer perception may be supported from tangible knowledge of network process management.

SYSTEMATIC REVIEW: NEUTROPHILS AND CLINICAL OUTCOMES IN PATIENTS WITH ACUTE CORONARY SYNDROMES AND/OR CARDIAC REVASCULARIZATION

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Background. Recent data of literature suggest that total white blood cell (WBC) count may be considered as independent prognostic factors in patients with cardiac damage due to acute coronary syndromes (ACS) and/or after cardiac revascularization by percutaneous coronary intervention or coronary artery bypass grafting surgery. However, a role of neutrophils in cardiovascular disease is less compelling. Therefore, we conducted a systematic review of the literature with the aim of identifying all the available evidence to clarify the role of neutrophils (absolute or relative count, neutrophil/lymphocyte ratio) as a prognostic risk factor in patients with ACS and/or cardiac revascularization.
Methods. All published studies evaluating the role of neutrophils as a risk factor for clinical outcomes were assessed using the MEDLINE and EMBASE databases. Study selection, data extraction and validity assessment was performed independently by two reviewers.
Results. Twenty-one studies (17 of which with positive results) for a total of more than 34000 patients were included. Ten of 13 studies in ACS patients found that neutrophils measured on-admission are related to mortality rate and/or to major adverse clinical events. A predictive value of neutrophils after cardiac revascularization procedures was reported in 7 out of 8 studies. Most of the studies showed that neutrophils were independent predictors of cardiovascular outcomes when analyzed concomitantly with WBC.
Conclusions. The findings of our systematic review highlight the potential application of this inexpensive and readily available inflammatory marker for risk stratification in patients with ACS and/or cardiac revascularization.

HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: GENETIC CHARACTERIZATION OF A CARDIOVASCULAR HIGH-RISK FAMILY

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Introduction. Familial Hypercholesterolemia (FH), a genetic disorder with autosomal dominant inheritance, is characterized by high plasma levels of LDL-cholesterol (LDL-c), with tendinous and skin xanthomas, corneal arch and a high prevalence of early cardiovascular disease. Case report Two brothers and two sisters were admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples. One brother had high basal LDL-cholesterol (LDL-c) levels (603 mg/dL), history of early myocardial infarction, severe carotid artery atherosclerosis, regularly treated by LDL apheresis and statin therapy. Both his children presented elevated LDL-c values (>300 mg/dL) and Achilles tendons xanthomas. The other brother showed a basal LDL-c of 362 mg/dL, Achilles tendons xanthomas and bilateral complete corneal arch. He had 2 children with the same phenotype. Both sisters had LDL-c of 290 mg/dL and 312 mg/dL respectively, tendinous xanthomas and were on therapy with ezetimibe/simvastatin. One daughter also showed the same phenotype. Genetic analysis for LDL-receptor mutation performed on 4 members of this family found an heterozygous mutation IVS15-3C>A. The functional assay was performed on LDL-receptor, indicating a residual activity of 52%.
Further genetic analyses are in progress to detect in the first male patient potential linkage polymorphisms. Discussion FH is caused by mutations of LDL-receptor (LDL-R), apo-B 100 genes or, as recently demonstrated by genetic family studies, non LDL-R/non apo-B, by mutations of PCSK9, a glycoprotein involved in LDL-R post-transcriptional homeostasis. Although the relationship between the different mutations of LDL-R and the clinical expression is yet not clear, the phenotypic expression of heterozygous FH is variable. The 2312-3 C→A mutation destroys the splicing acceptor site of intron 15, with deletion of the region of LDL-R transmembrane domain (p.Ala771_796del). The LDL-R functional study in these patients underlines a mean residual activity of 52%, which indicates the complete loss of function of the protein codified by mutated allele. Such mutation appears to be very common in our region and could provide a founder effect. According to previous studies, patients with 2312-3 C→A mutation present higher basal total-cholesterol values, prevalence of tendinous xanthomatosis and a mean max carotid IMT higher than patients with other kinds of LDL-R mutations, independently from gender, age, cholesterol-year score and primary cardiovascular risk factors. The presence of xanthomas is associated with a three times higher risk of cardiovascular disease in patient with FH, and is related to age. Patients with 2312-3 C→;A mutation are considered at high cardiovascular risk, so the early genotypic identification of these subjects could be a useful tool for an intensive and preventive therapeutic assistance.
Conclusions. Heterozygous FH is a disorder with a variable phenotypic expression. Future studies should investigate the relationship between phenotype and genotype and should identify high risk mutations in order to perform an effective cardiovascular prevention.

EPIDEMIOLOGY OF CARDIOVASCULAR DISEASES IN GENERAL PRACTITIONER POPULATIONS

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Cardiovascular diseases (CVD)is the leading cause of mortality,morbidity and hospitalization in both genders in all countries. It is so valid reason to implement programmes of prevention of these events. Smoking,physical inactivity,overweight and obesity, high blood pressure and high cholesterol levels indicate an urgent need to implement interest in terms of risk factors and events. General practitioner plays a crucial role in

this field because he is the first to evaluate this patients. For this reason,we make a study of our population,in collaboration with psychologist,and we had analysed all risk factors,studied the possible effects and tried to find a way of doing to improve quality of life of this patients. In this way we are sure to reduce the risk of develop cardiovascular diseases and decrease the costs for National Health Service.

EVALUATION OF THE EFFECT OF STATINS ON BLOOD PRESSURE CONTROL IN PATIENTS WITH HYPERTENSION IN A GENERAL PRACTITIONER'S POPULATION

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HMG-CoA reductase inhibitors (statins) are well known and widely used drugs for the treatement of dislipidemia. However,cardiovascular benefits of statins are only partially explained by their lipid-lowering power; pleiotropic effects of statins involve improvement of endothelial function, stability of atherosclerotic plaques,decrease of oxidative stress and inflammation,and inhibition of thrombogenic response.
Recent studies revealed a possible relationship between HMG-CoA reductase inhibitors use and blood pressure reduction,independently from cholesterol levels,probably explained by a stimulation of NO release from endothelial cells and other tissues. The same mechanism probably contribute to cardio and nephroprotective effects of some molecules of this class. An unequivocal demonstration of an antihypertensive effect of statins,however, is still lacking. General practitioner plays a crucial role in prevenction of high-prevalence diseases and is one of the largest prescriptors of lipid-lowering and antihypertensive drugs. Only few studies,as this time, analyzed the effect of statins on ambulatorial measured blood pressure. Our study aims to evaluate the impact of HMG-CoA reductase inhibitors on hypertension control in a general practitioner's population,which can represent a good model of "real world" population.

EFFECTS OF WHOLE GRAIN INTAKE ON CARDIOVASCULAR RISK FACTOR PROFILE IN SUBJECTS WITH METABOLIC SYNDROME: A 3 MONTH INTERVENTION

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Objective. To evaluate the effects of a 3-month dietary intervention using whole grain products on insulin and lipid metabolism in subjects with Metabolic Syndrome.
Methods. Sixty-one subjects of both genders, age 40-65 years, with Metabolic Syndrome participated in the study. After a 4-week run-in, participants were randomly assigned, according to a parallel design, to either an Experimental diet (whole grain products and low glycemic index) or a Control diet (refined cereal products and high glycemic index), each treatment lasting 3 months. The two diets had similar nutrient composition but the Experimental diet was richer in cereal fibre (32 vs 13 g/day) and had a lower glycemic index (42 vs 75). An intravenous glucose tolerance test and a standard test meal (composed of refined wheat or whole wheat products) were carried out at the beginning and at the end of the intervention.
Results. The Experimental and Control group were not different at run-in for BMI, waist circumference, fasting levels of glucose, cholesterol, HDL-cholesterol and triglyceride. These parameters did not change after 3 months of diet in both groups. At run-in, the Experimental and Control diet groups were not different for insulin sensitivity index (2.4±1.7 and 2.9±2.4) (M±SD) and insulin secretion. Again, these parameters did not change after 3 months in both groups. Plasma insulin and triglyceride responses to test meal, similar in the two groups at run-in, did not change after 3 months in the Control group while decreased respectively by 29% and 43% in the Experimental group (p=0.01 and p=0.04, respectively) with a significant difference between the two groups (p=0.05 and p=0.04, respectively).
Conclusions. Long term consumption of a diet based on whole grain products has not a relevant impact on insulin sensitivity in subjects with stable body weight; conversely, this type of diet improves significantly postprandial insulin and triglyceride metabolism.

CONJUGATED LINOLEIC ACID ISOMERS MIXTURE DOWNREGULATES TISSUE FACTOR EXPRESSION IN HUMAN MONONUCLEAR CELLS AND MACROPHAGES

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Background. The term conjugated linoleic acid (CLA) denotes a group of naturally occurring isomers of linoleic acid that differ in the position or geometry of their double bonds. The most predominant isomers in ruminant fats, c9, t11 (80%) and t10, c12 (20%), exert various beneficial effects including a decrease in proliferation, atherogenesis, diabetes and inflammation in animal models.
Tissue factor (TF), expressed mainly by infiltrating inflammatory cells, is considered one of the main contributors to the thrombogenicity associated with atheroma. In this study we investigated the effect of the blend of the two CLA isomers, c9, t11 and t10, c12, on TF expression in mononuclear cells (MNs) and macrophages (MØ).
Methods. MNs from peripheral blood of healthy donors and MØ, obtained by spontaneous differentiation of blood monocytes in culture, were incubated in the presence of blend with or without lipopolysaccharide (LPS). At the end of incubation supernatants were drawn, cells were disrupted by freezing and thawing, and procoagulant activity was assessed by a one-stage clotting time. TF and TNF-α; mRNA levels were measured by real time RT-PCR. TNF-α; levels were assessed by ELISA.
Results. The blend inhibited TF activity of LPS-stimulated MNs in a dose dependent way. Downregulation of TF activity was accompanied by a decrease in TF mRNA levels.
The decrease in TF activity was observed also in the presence of

other MNs agonist, namely IL1-β and TNF-α. Interestingly, TF inhibition was accompanied by a decrease in TNF-α mRNA level and TNF-α release respectively in MNs and MØ stimulated by LPS. When the two pure isomers c9, t11 and t10, c12 were tested, we observed a similar reduction in TF expression.
Conclusions. Our results suggest that CLA blend intake, by decreasing TF expression, could exert a beneficial effect on risk factors associated with the atherosclerotic process.

15-LIPOXYGENASE AND LIPOPROTEIN MODIFICATION: EFFECTS OF HDL3 ON ENDOTHELIAL ACTIVITY

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Background. HDL play a protective role in atherosclerosis by promoting the reverse cholesterol transport and by maintaining the endothelial function through the production of nitric oxide (NO). In this work we investigate the effect of HDL3- modified in vitro by 15-LO on the production of NO and on the activation and expression of endothelial nitric oxide synthase (eNOS) in endothelial cells.
Results. Incubation of endothelial cells with HDL3 resulted in increased eNOS phosphorylation (1,82±0,46 $p<0.05$ vs Ctrl), an effect which was lost upon 15LO-HDL3 incubation. This translated into an increased NO release from cells exposed to HDL3 (NO increases by about 4-time with 300 µg/ml of HDL3 compared to control cells), while, as expected, such effect was not observed in cells incubated with 15LO-HDL3. As SR-BI is the receptor involved in eNOS within the caveolae domain by HDL3, we investigated the effect of HDL3 and 15-LO-HDL3 on the expression of SR-BI, caveolin-1 (CAV-1) and eNOS in an endothelial cell line overexpressing SR-BI (EAhy-SR-BI). These cells present an elevated expression of SR-BI at both mRNA and protein level (mRNA increased by about 150-fold, and protein about 6-fold compared to wild type cells), resulting in a significantly higher interaction with HDL3. In this cells, under basal conditions eNOS mRNA expression is increased by approximately 2-fold compared to wild type cells, while no differences were observed in the expression of CAV-1 mRNA. Incubation of EAhy-SR-BI with HDL3 induced a further increase in the levels of CAV-1, SR-BI and eNOS mRNA, while this effect was lost when cells were incubated with 15-LO-HDL3.
Conclusion. Altogether these results suggest that in vitro modification of HDL3 with 15-lipoxygenase reduces the atheroprotective properties of HDL, indicating a possible role of this enzyme in the endothelial dysfunction, one of the earliest events in the development of atherosclerosis.

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED TO FAMILIAL COMBINED HYPERLIPIDEMIA

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Introduction. The Familial Combined Hyperlipidemia (FCH), the most frequent form of familial hyperlipidemia, consists in the increase of total cholesterol, triglycerides or both and has a polygenic and multifactorial basis not yet completely clarified. Genes encoding enzymes involved in lipid metabolism (Lipoprotein Lipase, Cholesteryl ester transfer protein, HMG-CoA reductase, Proprotein Convertase Subtilisin/Kexin-type 9) and apolipoprotein A5 (ApoA5) and C3 (ApoC3) are candidate genes for the association to FCH. The aim of this study is to perform the analysis of 15 Single Nucleotide Polymorphisms (SNP) in these genes in FCH patients in order to identify susceptibility markers for FCH.
Materials and Methods. We enrolled 50 patients with a clinical diagnosis of FCH and 138 healthy controls. After genomic DNA extraction from peripheral blood samples, the TaqMan assay was performed for the SNP typing. Data were analysed with PASW 18.0 software for the frequency comparison (Chi-square) and with Haploview 4.2 software for the allele count comparison and for the haplotype identification.
Results. At the comparison of genotype frequencies the variants S19W (c.56C>G; rs3135506) and -.1131T>C (rs662799) in ApoA5 gene were significantly associated to FCH disease ($p<0.02$ both). The allele count revealed differences in these 2 SNP and in the 2342G>C variant (rs5128) in ApoC3 gene ($p<0.02$, $p<0.01$ and $p<0.05$ respectively). The haplotype constituted by c.56C, -1131T in ApoA5 gene and 2342C in ApoC3 gene was significantly associated to FCH ($p<0.002$). The permutation test (100,000 permutations) confirmed this result ($p<0.004$).
Conclusions. Our data contribute to define the role of the variants S19W and -1131T>C in ApoA5 gene as genetic markers of FCH. Haplotype analysis revealed a risk haplotype for FCH susceptibility. Since the polygenic basis of the FCH disease, the combined study of different genetic variations allow to better characterize patients and to identify high-risk subjects.

EFFECT OF GREEN TEA SUPPLEMENTATION ON INSULIN SENSITIVITY AND ATP III METABOLIC SYNDROME COMPONENTS IN A COHORT OF NON DIABETIC WOMEN

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Introduction. It is well established that oxidative stress plays a role in insulin resistance and atherosclerosis progression. Epidemiological study suggest that high consumption of natural antioxidant has a protective role in cardiovascular disease. Green tea (GT) is rich in antioxidant such as epigallocatechin gallate. Aim Evaluate the effect of a green tea supplementation on insulin sensitivity and metabolic syndrome (MS) components, according to NCEP-ATPIII criteria, in a cohort of women.
Methods. 43 non diabetic women were enrolled for the study. At baseline (T0) patients underwent a physical examination, a body impedance analysis and a blood drawing. After a 1 month (T1), during which all received a normocaloric diet (NCD), patients were assigned to a NCD arm (20) or a NCD+GT arm (23). Subjects were thereafter evaluated 1 and 4 months (T3) later.
Results. 20 subjects completed the study: 7 in the NCD arm and 13 in the NCD+GT arm. Subjects not completing the study were not considered in the analysis. Due to asymmetrical drop out, subjects in the NCD arm had higher BMI and waist at T0. Similarly, metabolic score (number of MS components) was higher in NCD arm. During the follow up period NCD group show a significant reduction of BMI and waist while in the NCD+GT group we could only detect a reduction of waist at T3.
However, subjects in NCD+GT arm show a significant reduction of glucose levels and HOMA, not observed in the NCD arm. Similarly, metabolic score is significantly reduced at T6 in the NCD+GD arm but not in the NCD group. In a multivariate analysis GT consumption appears to inversely and independently correlate with metabolic score.
Conclusion. In our study GT supplementation seems to improve insulin sensitivity and reduce glucose levels and this effect appears to be independent from modification of body mass index.

ENDOTHELIAL DYSFUNCTION IS ASSOCIATED WITH ARTERIAL STIFFNESS IN HYPERTENSIVE PATIENTS WITH TYPE 2 DIABETES MELLITUS BUT NOT IN THOSE WITHOUT DIABETES

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We evaluated the relationship between endothelial function and aortic pulse wave velocity in essential hypertensive patients with (DM+) or without (DM-) type 2 diabetes. 46 DM+ with and 71 DM- matched for age, gender, blood pressure, hypertension duration and number and class of antihypertensive drugs were included. Brachial artery endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent dilation (25 µg sublingual glyceryl trinitrate, GTN) were assessed by high-resolution ultrasound and computerized edge detection system. Aortic pulse wave velocity (aPWV) was determined by applanation tonometry. DM+ had a lower FMD than DM- (3.1±1.7 vs. 5.0±3.1, p<0.0001) with no difference in GTN response. aPWV was higher in DM+ (10.0±1.9 vs. 8.8±1.3 m/s, p<0.0001). Considering FMD (4%) and aPWV (8.5 m/s) median values, the odds ratio (OR) for increased aPWV in the presence of reduced FMD was increased (2.31 CI95%: 1.01-5.44) upon adjustment for age, gender, systolic blood pressure (systBP) and body mass index (BMI), but not for diabetes (OR 2.09, CI95% 0.88-4.95). In DM+, aortic PWV was related to systBP (r=0.39), BMI (r=0.36, both p<0.01), log-triglycerides (r=0.36), and urinary albumin:creatinine ratio (r=0.37), but not with disease duration, fasting plasma glucose, HbA1c, lipid profile or log-hs-CRP. aPWV was related to FMD (r=-0.44, p=0.001), but not to GTN.
In multiple regression analysis only FMD (r2=0.17, p<0.001), systBP (r2=0.16,p<0.001) and BMI (r2=0.12, p<0.001) remained independent predictors of aPWV (full model: r2=0.50). In DM- aPWV correlated with age (r=0.36, p=0.003), BMI (r=0.27, p=0.02) and systBP (r=0.31, p=0.009) but not with other clinical and vascular parameters. aPWV was not related to FMD in these patients. Only age was related to FDM (r=-0.27) in both DM+ (p=0.04) and DM- (p=0.02). In conclusion, increased aortic stiffness is associated with endothelial dysfunction in hypertensive diabetic patients, but not in hypertensive normoglycemic subjects who have lower aPWV but less impairmed FDM. This association suggests a specific deleterious effect of diabetes on vascular function and structure.

EVALUATION OF POST-PRANDIAL VARIATION OF LIPID PROFILE AND INSULIN RESISTANCE BIOMARKERS AFTER AN ORAL FAT LOAD IN DYSLIPIDEMIC PATIENTS TAKING N-3 PUFAS: A RANDOMIZED, DOUBLE-BLIND, CONTROLLED STUDY

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Aim. To evaluate the effects of 6 months treatment with ω-3 polyunsaturated fatty acids (n-3 PUFAs) on lipid profile and insulin resistance biomarkers at the baseline, and after an OFL in patients affected by combined dyslipidemia.
Materials and Methods. A total of one hundred and sixty-seven patients affected by combined dyslipidemia were enrolled in the study; patients were assigned to receive, as addition to diet and physical activity, placebo or n-3 PUFAs 1 g three times a day, during the meals, for 6 months. We evaluated at the baseline, and after 2, 4, and 6 months these parameters: body mass index (BMI), body weight, fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), blood pressure, lipid profile, resistin (r), retinol binding protein-4 (RBP-4) adiponectin (ADN), visfatin. Furthermore at the baseline and at the end of the study

all patients underwent an euglycemic hyperinsulinemic clamp to evaluate M value, and total glucose requirement (TGR) and an oral fat load.
Results. Regarding the parameters in a baseline condition, we observed a better decrease of Tg, and a better increase of HDL-C, and ADN with n-3 PUFAs compared to placebo; regarding other insulin resistance biomarkers, instead, there was a decrease of r, and RBP-4 after the treatment with n-3 PUFAs not observed with placebo, but no differences were observed in group to group comparison. Regarding the situation after the OFL, and comparing the OFL performed at the baseline and at the end of the study, there was a decrease of Tg, r, and RBP-4 values, and an increase of ADN value with n-3 PUFAs, but not with placebo.
Conclusions. Treatment with n-3 PUFAs gave a better improvement of lipid profile and ADN compared to placebo, both in a baseline condition and after an OFL.

EFFECTS OF ANTI-TNF-ALPHA THERAPY ON SERUM LIPID PROFILE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background. Patients with severe rheumatoid arthritis (RA) present an increased cardiovascular risk, frequently linked with an atherogenic lipid profile. The patogenetic mechanism has not already been completely explained, but some pro-inflammatory cytokine are probably involved (TNF-alpha et al.). AIM OF THE STUDY: to evaluate the effects of biologic therapy on lipid profile in comparison with conventional therapy, in patients with rheumatoid arthritis.
Methods. 94 patients with RA treated with anti TNF-alpha drugs (Etanercept, Adalimumab and Infliximab) have been compared with 57 patients (matched control group) treated with traditional DMARDs. We evaluated lipid profile - using conventional methods - and clinical evolution of illness, using the "DAS 28" index.
Results. In patients treated with biological drugs, we observed a reduction of total-cholesterol concentration by 5,0%, of LDL-cholesterol by 11,7% and an increase of HDL-cholesterol by 8,8%, with a reduction of the atherogenic ratio from 3,63 to 3,17 (-12,6%). In the control group we observed a reduction of total-cholesterol by 3,73%, of LDL-cholesterol by 11,1% and an increase of HDL-cholesterol by 10,7%, with a reduction of the atherogenic ratio from 3,72 to 3,23 (- 13,2%).
Conclusions. The effects on lipid profile and DAS 28 index were comparable between the group with RA treated with biologic agents and DMARDs drugs, even if the severity of AR was higher in the former.

TRANSCRIPTOMIC ANALYSIS OF MACROPHAGE POLARIZATION: ROLE OF PPARγ IN ALTERNATIVE ACTIVATION

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Background. Macrophages are an heterogeneous cell population that plays a central role in atherogenesis. Classical macrophage activation (M1) induced by IFNγ and LPS, exerts inflammatory activities, while alternative activation (M2) is promoted by IL-4 and/or IL-13 and shows immunoregulatory properties. PPARγ is a nuclear receptor with anti-inflammatory properties through negative modulation of nuclear factor NF-kB, and seems to be implicated in macrophage activation.
Aim. Evaluation of the biological processes involved in the different type of macrophage activation compared to the effects induced by PPARγ on this cell population. Methods. Human macrophages were stimulated with INFγ and LPS (M1), IL-4 (M2) and PPARγ-agonist (GW1929, Sigma). Resting macrophage (no stimuli) were use as a control. A transcriptome analysis using whole genome dual color microarray technology (Agilent technologies) was applied for each condition. Gene ontology (GO) and pathway analysis was performed using GOrilla (http://cbl-gorilla.cs.technion.ac.il/)and David Functional Annotation Tools (http://david.abcc.ncifcrf.gov/).
Results. Comparing PPARγ-agonists stimulated macrophages with M2 macrophages, we observed an increase in cell cycle activity (GO terms: "cell-cycle" :p-value =2.2E-15, "M-phase" p-value =6.1E-13, "DNA-replication": p-value =6.8E-11; KEGG pathway: "cell cycle": p-value =4.5E-7) and a slight reduction in immunoregulatory processes (GO terms: "cell-cell signaling": p-value =2.15E-4; KEGG: "cytokine-cyotokine receptor interaction": p-value =3.04E-4) induced by PPARγ-agonist. As expected, the differences appear more significant comparing PPARγ with M1: the p-value of up-regulated inflammatory processes and immune response in M1 are about E-30 for GO and E-10 for KEGG. Up-regulation of cell-cycle pathways is confirmed comparing PPARγ-agonist-stimulated macrophages with resting macrophages.
Discussion. Addition of PPARγ-agonist induces an activation more similar to M2 than M1, due to its anti-inflammatory properties, as previously described (Bouhel et al.2007). Anyway, PPARγ-agonist activation reduces inflammatory cytokine production and increases cell-cycle gene expression respect to M2, suggesting a role of PPARγ in the regulation of macrophage differentiation.

EFFICACY OF LIFESTYLE INTERVENTIONS IN TACKLING METABOLIC ABNORMALITIES ASSOCIATED WITH ANTIRETROVIRAL THERAPY IN HIV PATIENTS

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Introduction. HIV prognosis has dramatically changed after introduction of antiretroviral therapy. Treated patients present metabolic abnormalities, recalling metabolic syndrome, which confer an higher cardiovascular risk. Retinol-binding-protein4 (RBP4) has been shown to be involved in glucose metabolism but its role in HIV has not been established.
Aim. Test the efficacy of a therapeutic-lifestyle-change (TLC) or generic dietary counseling (GC) in correcting metabolic abnormalities seen in HIV patients. Secondary aim was to evaluate RBP4 in HIV patients receiving different treatments. Methods 50 HIV patients were enrolled. The protocol was approved by the local Ethics Committee. At baseline (T0) patients underwent a physical examination, a body impedance analysis and a blood drawing. According to antiviral regimen, subjects were divided into 3 groups: naïve (13), receiving Non-nucleoside reverse transcriptase inhibitors (NNRTI) and Nucleoside reverse transcriptase inhibitors (NRTI)(22) and receiving NRTI+protease inhibitors (NRTI/PI) (14). Patients in each group were subsequently assigned to receive GC or TLC (ATP III like)and followed for 6 months (T6). RBP4 levels were determined with a commercial ELISA kit.
Results. At T0, patients in the 3 groups did not differ in BMI, waist, blood pressure, lipid profile nor glucose nor insulin. After lifestyle intervention no significant changes were detected. Metabolic score (number of metabolic syndrome components) did not change after intervention. Effects were similar with both GC and TLC. At T0, RBP4 levels positively correlates with cholesterol (r=0.42, p=0.02) and triglycerides (r=0.48, p=0.001)and negatively with HDL- Chol (r=-0.025, p=0.05) while no correlations were observed with glicaemia, insulinaemia nor HOMAindex.
Conclusion. Our data show that a midterm lifestyle intervention in HIV patients is not effective in tackling metabolic abnormalities associated with antiretroviral therapy, suggesting a more aggressive strategy is needed. RBP4 levels do not predict insulin sensitivity suggesting a marginal role of this protein in glucose homeostasis alteration seen in HIV patients.

IDENTIFICATION OF A NOVEL MUTATION OF MTP GENE IN A PATIENT WITH ABETALIPOPROTEINEMIA IN IRAN

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Introduction. Abetalipoproteinemia (ABL), or Bassen-Kornzweig syndrome, is a rare autosomal recessive disorder of lipoprotein metabolism, characterized by fat malabsorption, hypocholesterolemia retinitis pigmentosa, progressive neuropathy and acanthocytosis from early infancy.
Methods and Results. We describe the clinical and molecular characterization of a 6-month-old infant born of consanguineous, apparently healthy parents from Iran. The proband was hospitalized because of failure to thrive, greasy stool and vomiting. The patient's serum lipid profile, the clinical phenotype and the duodenal histology suggested the clinical diagnosis of ABL. The MTP gene analysis by direct sequencing revealed a novel homozygous mutation (c.1586 A > G – H529R). The parents were heterozygotes for the same mutation and interestingly the father showed a lipid profile characterized by a slight reduction of total and LDL-cholesterol plasma levels
Conclusion. In conclusion we have described a novel missense mutation in MTP gene, the H529R, in a iranian child with an ABL phenotype.

A CASE OF NO-REFLOW PHAENOMENON IN A SYSTEMIC LUPUS ERITEMATOSUS PATIENT

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Introduction. Inflammatory and autoimmune diseases suffer from increased cardiovascular morbidity and mortality owing to accelerated atherosclerosis and premature coronary artery disease. Most of the mortality of Systemic Lupus Eritematosus (SLE) patients is nowadays attributed to premature coronary atherosclerosis. Beyond the epicardial coronary disease, also coronary microvascular disfunction (CMD) may worsen the outcome.
Materials and Methods. We present the case of a 32-year-old woman who suffered from systemic lupus erytematosus for more than 12 years, resulted in diffuse vasculitis and end stage renal failure in haemodialitic therapy three times a week. She underwent kidney transplant and explant twice due to severe rejection. After a percutaneous coronary intervention for an acute coronary syndrome she presented a no-reflow phaenomenon (NRP) which caused cardiogenic shock.
Discussion. NRP is associated with CMD, which is responsible for the inability of a previously ischemic region to be reperfused. NRP is caused by functional and structural damage of coronary microvasculature during ischemia. This damage is aggravated when the culprit vessel is reperfused. Different pathogenetic mechanism predispose to NRP. Recent studies demonstrated that CMD can be detected in patients affected by chronic inflammation disorders such as SLE. During reperfusion of ischemic myocardium, preservation of the coronary microvasculature is essential to the ultimate recovery of myocardial function. In this condition CMD refers to the impairment of resting blood flow within the post-ischemic vasculature.
The clinical significance of microvascular dysfunction lies in its association with worse cardiovascular outcomes. Previous studies of SLE patients found lower survival and a higher frequency of reintervention when compared with the general population. No specific mechanisms have been identified, but this higher occurrence of cardiovascular adverse events in SLE-patients could be due both to epicardial disease and CMD.
Conclusion. Vascular inflammation in SLE may result in unexpected extensive coronary no-reflow phaenomenon following an acute coronary syndrome.

MULTIDETECTOR COMPUTED TOMOGRAPHY (MDCT): ABLE TO DEFINE THE PLAQUE COMPOSITION AS ASSESSED BY HISTOLOGY?

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Background. Plaque morphology is an important predictor of stroke risk. Although plaque morphology is not used, so far, in the decision making of whether to perform carotid endoarterectomy (CEA) or not, having a non-invasive technique capable of diagnosing vulnerable plaque would be very useful in the recognition of patients at high risk of cerebrovascular disease.
Aim. To compare human carotid plaques composition obtained by means of histological analysis and MDCT.
Methods. 37 patients (22 male, 15 females; mean age: 70±7 years) undergoing CEA due to high grade carotid artery stenosis (82±9%) were evaluated with MDCT for non-invasive plaque morphology assessment prior to CEA. Plaques removed during surgery were divided into 5 mm pieces.
The part corresponding to the point of maximum stenosis was formalin fixed and paraffin embebbed for histology. Sections were stained with Haematoxilin-Eosin and Masson-Trichrome to assess plaque morphology and composition (collagen, smooth muscle cell, lipids and calcium). Kappa statistics was used for the degree of agreement between the histological and MDTC images.
Results. Images of histological sections were computer-reconstructed with Zeiss- Panorama software after being photographed under a microscope (90 frames for each section on average). The area occupied by collagen, smooth muscle cells, lipids, calcium and thrombus was then quantified (absolute amount, mm^2, and percentage over the total area) using the Zeiss-Axiovision-measurement software. According to the composition thus obtained, plaques were classified into four classes: lipidic (10%), fibrotic (45%), mixed (35%), and calcific (10%). DSCT plaque analysis was performed on a image of the vessel area at the highest degree of stenosis. A good correlation between histology and MDCT was found (k=0.76).
Conclusion. MDCT angiography of the carotid arteries is feasible and the evaluation of carotid plaque composition allows non-invasive assessment of different plaque components. This may have an impact on the non-invasive differentiation of vulnerable plaques.

GENETIC DIAGNOSIS IN PRIMARY SEVERE HYPOBETALIPOPROTEINEMIA. A MOLECULAR CONUNDRUM

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Background. Severe hypobetalipoproteinemia (HBL) was reported in homozygous familial hypobetalipoproteinemia (FHBL), abetalipoproteinemia (ABL) and chylomicron retention disease (CRD). Because of the variable clinical expression and uncertain transmission, the diagnosis of these disorders relies on the analysis of the candidate genes: APOB, MTP, SARA2 and PCSK9.
Methods and Results. We investigated 10 adults (19-52 ys.) and 23 children (5 months-14 ys.) with severe HBL. Plasma LDL-C ranged from <3 to 25 mg/dl and apoB from <2 to 61 mg/dl. Clinical manifestations were fatty liver and steatorrhea (in the adults) and growth retardation, severe steatorrhea, and enterocyte fat accumulation (in children). Mild neurological symptoms and/or retinitis pigmentosa were observed in two patients. In all patients MTP gene was analysed first. Five children were found to be homozygous for mutations in MTP gene resulting in truncated MTPs. An adult patient was found to carry a splice site mutation, resulting in a MTP protein with an in-frame deletion of 36 amino acids, and another mutation not yet identified. In the 27 subjects negative for MTP mutations we analysed the APOB gene.
Five subjects were homozygous or compound heterozygous for mutations resulting in short apoB truncations. Ten subjects were simple heterozygotes for mutations resulting in short truncated apoBs (<apoB-30). In the remaining 12 subjects no mutations in APOB, PCSK9 and SARA2 genes were found.
Conclusions. This study confirms that in severe HBL the differential diagnosis between ABL and FHBL relies on the analysis of candidate genes, especially when no information on the transmission of the trait is available. Subjects homozygous for MTP and APOB gene mutations have more severe and earlier clinical manifestations than heterozygous carriers of truncated apoBs. The observation of 12 patients with no candidate gene mutations suggests the existence of mutations located in still unknown genes.

TANGIER DISEASE: A LIPID DISORDER MORE FREQUENT THAN COMMONLY BELIEVED

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Background. Loss of function mutations ABCA1 gene in homozygosity/compound heterozygosity are the cause of Tangier Disease (TD), an exceedingly rare disorder characterized by extremely low levels of HDL-C and apoA-I, accumulation of cholesteryl esters in various organs, associated with a variety of clinical manifestations. Objective. Within the frame of the study of monogenic disorders of HDL metabolism, our objective was to investigate at clinical and molecular level novel patients suspected to have TD.
Methods. Sequencing of ABCA1 gene, in silico and wet analysis of ABCA1 transcripts, cholesterol efflux in culture skin fibroblasts.
Results. Proband NP was a 41 yrs male with thrombocytopenia, mild splenomegaly and asymptomatic coronary atherosclerosis, whose identical twin brother had died from acute MI. Both subjects had facial dysmorphic features and undetectable HDL-C

and apoA-I. They were found to be compound heterozygtotes for novel ABCA1 mutations (c.1758 ins G, M586>Fs629X and H1600R). No ABCA1 mediated cholesterol efflux was found in cultured fibroblasts.
Proband CJ a 74 yrs female with undetectable HDL-C, mild thrombocytopenia and splenomegaly, but no CHD, was found to be heterozygous for a previously reported mutation (D1099Y); however despite a single mutation, the ABCA1 mediated cholesterol efflux in her fibroblasts was similar to that found in other molecularly defined TD patients.
Proband ML was a 6 yrs female with undetectable HDL-C and apoA-I, hepato-splenomegaly, anemia and foam cells in bone marrow. Similar features were present in her 25 yrs old sister. Both siblings were found to be homozygous for a novel nucleotide substitution in intron 31 (IVS31-34 a>g). By in silico analysis this mutation was found to introduce a new acceptor site in IVS31, resulting in an abnormal mRNA containing part of intron 31 and predicted to encode a truncated ABCA1.
Proband AA a 37 yrs male with undetectable HDL, yellow tonsils and MI, was found to be homozygous for a novel nucleotide substitution in intron 20 (IVS20-2A>C). This mutation caused a splicing defect resulting in the formation of three abnormal mRNAs predicted to encode truncated proteins devoid of function.
Conclusions. This series of patients added to those we reported previously suggests that TD is not as rare as previously assumed. The association of very low HDL-C and apoA-I levels, with otherwise unexplained splenomegaly with moderate thrombocytopenia or anemia are frequent phenotypic manifestations suggesting the diagnosis of TD.
The reason why only a subset of patients has severe premature CHD still remains elusive. Apparently the susceptibility to CHD is not related to the type of ABCA1 mutation or the defect in cell cholesterol efflux. Finally, some TD patients may have dysmorphic features.

CHOLESTEROL EFFLUX POTENTIAL OF SERA FROM PATIENTS BEFORE AND AFTER LDL-APHERESIS

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Efflux of free cholesterol (FC) from peripheral cells to serum is considered a critical step in the reverse cholesterol transport (RCT) pathway. The efficiency of whole serum to promote cellular cholesterol efflux depends on the ability of individual lipoproteins, especially HDL fractions, to interact with specific transporters involved in the efflux pathways and to act as extra cellular acceptors. Low density lipoprotein (LDL)-apheresis is accepted as the treatment of choice to reduce serum cholesterol in patients with homozygous familial hypercholesterolemia (FH) and in heterozygotes with cardiovascular disease refractory to lipid-lowering drug therapy. LDL-apheresis may dramatically affect lipoprotein profile by lowering, in addition to LDL, triglycerides and HDL. The aim of our study was to quantify the efflux potential of sera from patients before and soon after LDL-apheresis; serum was obtained from 15 patients with FH who have been received bi-weekly LDL-apheresis. The capacity of sera to promote cholesterol efflux was tested in pathway-specific cell models for passive diffusion (PD), ABCA1-, ABCG1- and SR-BI-mediated efflux. Cells were labeled with [3H]-cholesterol and then equilibrated in 0.2% BSA-containing medium. Cholesterol efflux to sera was measured by the release of [3H]-cholesterol into the medium and calculated as percentage of total cell radioactivity. The LDL-apheresis treatment reduced total cholesterol and triglycerides by 60% to 70% and HDL by 20%. The LDL-apheresis reduced the ability of sera to promote cholesterol efflux by SR-BI and passive diffusion by 18.2% (±1.3%; $p<0.001$) and 23% (±1.3%; $p<0.0001$), respectively. In addition the ABCA1-mediated process decreased by 24% (±1.8%; $p<0.0001$). Within 48 hours HDL, but not LDL, plasma concentrations return to baseline values and in parallel the capacity of sera to promote cholesterol efflux return to normal levels, indicating that LDL-apheresis only temporarily impairs the serum ability to promote cell cholesterol efflux. These results also suggest that HDL are the main lipoproteins that modulate serum efflux potential process.

IS COAGULATION CASCADE BALANCE, ASSESSED BY THROMBOPATH®, ASSOCIATED WITH PLATELET HYPER-REACTIVITY?

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Hypercoagulability and platelet hyper-reactivity are involved in atherothrombotic diseases and are associated with an increased risk of the occurrence of cardiovascularevents in acute coronary syndrome (ACS) patients undergoing PCI with stent implantation. Thrombin generation represents a potent stimulus able to induce platelet activation and to influence anticoagulant protein C/protein S pathway. Thrombopath® is a global assay sensitive to PC system and toprothrombotic factors such as high levels of FII and FVIII.No data are available on the Thrombopath® determination in high risk cardiovascular patients and its association with platelet hyper-reactivity.
Our aim was to evaluate the association between coagulation cascade balance, assessed by Thrombopath®, and platelet function after acute coronary events in 157 (118 males/39 females) ACS patients undergoing PCI with stent implantation.
Thrombopath® analysis was performed on ACL Top (Instrumentation Laboratory). Platelet function was evaluated by VerifyNow Aspirin (ARU) and VerifyNow P2Y12 (PRU) (Accumetrics). All patients received 600 mg clopidogrel loading dose followed by 75 mg daily and aspirin 100 mg daily for 6 months. High-on treatment platelet reactivity (HPR) by VerifyNow was defined by the presence ofPRU ≥240 or ARU ≥550. Altered Thrombopath (ThP)values were defined on the basis of mean minus2SD of the results obtained in control population (n=150) and was set at 77.31%protac-induced coagulation inhibition (PICI).
PICI % values were significantly ($p<0.05$) lower in patients with HPR by VerifyNow P2Y12 than in patients without HPR [76.63 (40.82-90.75) vs 80.01 (45.60-94.12) PICI %]. Similarly, in patients with HPR by VerifyNow P2Y12 or HPR by VerifyNow Aspirin we found significant ($p<0.05$) lower levels of PICI % [76.45

(40.60-90.13) vs 80.21 (45.64-97.15) PICI %]. Correlation analysis showeda slight, but significant, correlation between PRUand PICI % values (r=0.19, p=0.045). Among the 62 ACS patients with HPR by VerifyNow P2Y12 (PRU≥240) we found the occurrence of altered ThP values in 35 (56.5%) patients, whereas in patients without HPR the percentage of patients with altered ThP values was 31.8% (27/85)(p=0.031). Altered ThP values were found in 10/18 (55.6%) ACS patients with HPR by VerifyNow Aspirin (ARU≥550) and in 63/139 (45.2%) patients without HPR.
ACS patientswith HPR by VerifyNowP2Y12 and/or HPR by VerifyNow Aspirinshoweda significant (p=0.010) higher prevalence (58.3 %, 42/72) of altered PICI valueswith respect to patients without HPR (36.5%, 35/85).
Our results indicate, for the first time, the association between unbalanced coagulation pathway and platelet hyper-reactivity in ACSpatients. The unbalance between anticoagulant and procoagulant factors, assessed by Thrombopath® assay, may represent a mechanism underlying platelet activation.

SERUM GLUCOCORTICOID INDUCIBLE KINASE(SGK)-1 AND ENDOTHELIAL DYFUNCTION: MODULATION OF INSULIN ACTION AND OXIDATIVE STRESS

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Introduction. Endothelial dysfunction has a crucial role in development of diabetes vascular complications. Oxidative stress can explain the increase of atherosclerosis in diabetic patients. Diabetic hyperglycemia causes endothelial dysfunction, mainly decreasing endothelial Nitric Oxide Synthase (eNOS) activation and subsequently nitric oxide (NO) production. Hyperglycemia can activate different damaging cell pathways increasing cell apoptosis, reactive oxygen species (ROS) levels and diminishing Na+-K+ ATPase activity. In our study we analyzed the role of Serum Glucocorticoid Kinase (SGK)-1, a member of serine /threonine kinase family, in insulin mediated eNOS activation and NO production in basal conditions and after high glucose (HG) and glucosamine treatments.
Materials and Methods. pLPCX vectors have been used to generate retroviruses to infect Human umbilical endothelial cells (HUVECs) with SGK-1, SGK-1 lacking of the N-60 amino acids (Δ60) and SGK-1Δ60 kinase dead constructs, than we analyzed infected cells with western blot, immunofluorescence and flow cytometric methods.
Results. We found that SGK-1 activity is increased in SGK-1Δ60 infected endothelial cells stimulated or not with insulin. These cells have an increased activity of Na+-K+ ATPase and eNOS. Moreover these cells have reduced ROS production and lower apoptotic levels respect to other infected cells. Expression of PKG, Akt-1 and SGK-1 m-RNA has been measured in diabetic and non diabetic atherosclerotic plaques. Expression of SGK-1 mRNA is higher in diabetic compared with not diabetic atherosclerotic plaques.
Conclusions. In endothelial cells SGK-1 reduces oxidative stress levels, improves cell survival and restores insulin ability to increase eNOS mediated NO production after HG and glucosamine stimuli. Furthermore we found that SGK-1 mRNA levels were over-expressed in diabetic plaques. We can conclude that SGK-1 is involved in the pathophysiology of diabetic atherosclerotic syndrome and SGK-1 can improve endothelial cell function and survival.

GENDER DIFFERENCES IN THE PREVALENCE AND CONTROL OF CARDIOVASCULAR DISEASE RISK FACTORS AMONG PATIENTS WITH TYPE 2 DIABETES: RESULTS FROM THE MULTI-FACTORIAL INTERVENTION IN TYPE 2 DIABETES IN ITALY (MIND.IT) STUDY

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Introduction. The general decline in cardiovascular (CV) mortality observed in the general population and in diabetic men has not been observed in women with diabetes. The study explores CV disease risk factors prevalence and control in men and women with type 2 diabetes and the impact thereon of obesity and treatment approach.
Materials and Methods. A cross-sectional survey was conducted at 10 hospital-based outpatients diabetes clinics.
Results. 1297 men and 1168 women without previous CV events were enrolled. Women were older and smoked less than men. BMI and prevalence of abdominal obesity were higher in women, as well as HbA1c, LDL cholesterol, non-HDL cholesterol, systolic blood pressure (SBP) and serum fibrinogen. Accordingly optimal targets for HbA1c (<7%), LDL cholesterol (<100 mg/dL), HDL cholesterol (>40 (men) or >50 (women) mg/dL), and SBP (<130 mmHg) were less frequently achieved by women than men (respectively 33.8% vs 40.2%; 14.6% vs 19.2%; 34.1% vs 44.5%; 68.8% vs 72; p<0.05 for all).
Findings were confirmed after stratification for waist circumference, thus suggesting that gender differences are not completely accounted for by a higher prevalence of obesity/abdominal adiposity in women. As for treatment, women were more often than men on insulin treatment and antihypertensive medications, while no differences were observed in lipid-lowering drugs use, anti-platelet treatment was more frequent in men.
Conclusions. CV disease risk factors are more prevalent and less well controlled in women than in men. Gender disparities cannot be fully explained by differences in abdominal obesity, or intensity of treatment. Other factors, maybe hormonal influences and/or a reduced response to pharmacological treatments should be more deeply explored. The differences in CVD risk factors could partially explain the high CV morbidity and mortality in diabetic women.

ROLE OF CLASS I HISTONE DEACETYLASES IN DIABETES

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Because histone deacetylases (HDACs) are involved in the determination of skeletal muscle fiber type, in this study we aimed at investigating whether epigenetic mechanisms also control metabolic pathways at the transcriptional level. To this end, we examined the metabolic effects of biochemical inhibition of class I and class II HDACs in skeletal muscle and the underlying mechanisms. Staining of myotubes treated with HDAC inhibitors (HDACi) showed an increase of mitochondrial density and activity. Moreover, transcriptome analysis revealed an increase of OXPHOS genes and of genes encoding fatty acid and glucose metabolism. In Db/Db obese and diabetic mice we observed that treatments with HDACi reduce glycemia, plasma insulin level and improves glucose clearance. In vivo metabolic study revealed an increase of oxygen consumption and a net decrease of the respiratory exchange ratio, implying a more oxidative metabolism. Collectively, our results suggest that HDACs play an unexpected role in energy metabolism and may represent key regulators in diseases based on metabolic alterations.
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LOXIN POLYMORPHISM IS ASSOCIATED WITH INCREASED RESISTIN LEVELS AND WITH OXIDATIVE STATUS

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Background. LOX-1 is a type II membrane protein belonging to the C-type lectin family of molecules and it is encoded by OLR1 gene. A new functioning splicing of the OLR1 gene lacks exon 5 and it is named loxin. Levels of loxin mRNA and protein expression were negatively associated with the incidence of myocardial infarction in humans. Loxin has not the site for the ligand binding, but interacts with the full-length LOX-1 receptors blocking their cellular expression, OxLDL binding activity and its uptake.
Methods. Three hundred men were randomly selected from the original cohort invited to participate in a metabolic screening in 2001-2003 and contacted for a follow-up visit from January to November 2008. Measurements of glucose, insulin, total cholesterol, HDL-cholesterol triglycerides, high-sensitivity C-reactive Protein, nitrotyrosine, total antioxidant status (TAS), resistin levels and IVS4-14 A>G polymorphism of human gene OLR1 (loxin) were performed.
Results. The IVS4-14 A>G genotypic distribution in this population was in Hardy-Weinberg equilibrium and the G allele frequency was 0.37. The mean resistin and nitrotyrosine values were significantly higher in homozygous subjects for the variant G allele, whereas TAS was at the lowest values. In a multiple regression model, resistin and nitrotyrosine values were significantly and directly associated with G allele, while TAS was inversely associated. This association was still kept significant when multiple regression analysis was adjusted for age, BMI, smoking habits, baseline diabetes, hypertension and coronary artery disease.
Conclusion. The finding that subjects carrying the G allele have higher circulating levels of resistin gives support for a major uptake of Ox-LDL by macrophages, smooth muscle cells, and monocytes. Ox-LDL binding to LOX-1 enhances reactive oxygen species through the activation of a membrane-bound NADPH oxidase decreasing blood antioxidants status. Loxin polymorphism is likely to play a role in the early stages of the atherosclerotic process.

A CASE OF CEREBROTENDINOUS XANTHOMATOSIS IN A WOMAN WITH A NORMAL CHOLESTEROLEMIA

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Introduction. Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive inborn error of bile acid synthesis caused by a deficiency of cytochrome P450 sterol 27-hydroxylase (CYP27) which impairs the conversion of cholesterol to cholic and chenodeoxycholic acids. CTX patients show increased levels of total cholesterol and cholestanol, and decreased HDL levels. Incomplete oxidation of cholesterol side-chain leads to excretion of large amounts of urinary 25-hydroxylated-C27-bile alcohols. We report a 29-year-old female with CTX showing bilateral xanthomas in elbows and Achilles tendons, cataract, and mental retardation. Brain MRI does not show lipid accumulation.
Materials and Methods. Lipid profile was determined by enzymatic methods. LDL subfractions were separated by polyacrylamide gel tube electrophoresis. Sterol profile analysis in plasma and red blood cell membranes (RBCM) was performed by gas chromatography (GC-FID) and mass spectrometry (GC-MS). Urinary bile alcohols were measured by liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS). For the genetic analysis the 9 exons of the CYP27 gene were amplified by PCR and directly sequenced.
Results. Lipid profile was normal, while small dense LDL values were 29% (r.v.<10%). Cholestanol in plasma and RBCM were at least 100 times higher than controls. Sterol profile also showed increased levels of zymostenol, 7-dehydrocholesterol, and lathosterol. Urinary bile alcohols, such as tetrahydroxy-, pentahydroxy-, and hexahydroxy, were 20-100 times higher than controls. At the sequence analysis the mutation IVS7+5 was found at the homozygous status. This mutation causes the skipping of exon 7 leading to a frameshift and to a truncated protein.
Conclusions. In this patient, although cholesterol level was normal, cholestanol and other metabolites were elevated even though brain lipid accumulations were not observed. Furthermore, the increased levels of small-dense LDL could promote a faster lipid accumulation in tendons and in arterial walls. The molecular study of parents in comparison with LC-ESI-MS/MS analysis is in progress.

CREATININE LEVELS AND INTIMA MEDIA THICKNESS IN A POPULATION BASED COHORT STUDY: FINDINGS FROM PROGETTO ATENA

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The relationship between Creatinine (Cr) levels and common carotid intima-media thickness (IMT) has been evaluated in a population-based cohort study in women, aged 30-69, living in the metropolitan area of Naples, Southern Italy (Progetto ATENA). Serum Creatinine, Cholesterol, HDL-cholesterol, LDL-cholesterol, Triglyceride, Glucose, Insulin and HOMA were measured in 310 women, as a part of 5.062. In this group carotid ultrasound examination (B-Mode imaging) was performed and IMT was calculated. Women were classified by Cr levels, group 1: Cr > 1mg/dL; group 2: Cr <1 mg/dL. Group 1 and group 2 women differ significantly (p<0.001) only in Systolic Pressure values (156.8 vs 139.1 mm/Hg). 64.0% of women in group 1 have Cr clearance, estimated by Cockroft e Gault formula < 60 ml/min.
Multivariate analysis showed a significant association between common carotid IMT >1.2 mm (90° percentile of studied population) and Cr levels in group 1 after controlling for age, and BMI (OR 4.2; 95% CI 1.24 -14.46; p=0.021 for group 1 Cr; or age, and Metabolic Syndrome (OR 3.98; 95% CI 1.15 -13.78; p=0.029 for group 1 Cr). These findings showed the independent relationship between Creatinine and Common carotid IMT in this population of Mediterranean women, independently of age, BMI and Metabolic Syndrome.

RELATION OF BODY MASS INDEX WITH CAROTID INTIMA-MEDIA THICKNESS AND DIAMETERS IS INDEPENDENT OF METABOLIC SYNDROME IN POST-MENOPAUSAL MEDITERRANEAN WOMEN

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Aim. To evaluate if overweight and obesity were associated with arterial abnormalities in post-menopausal (p-m) women and the contribution of metabolic syndrome.
Patients and Methods. Three hundred and ninety p-m women (mean age 63.1±7.7 years), living in the metropolitan area of Naples, Southern Italy, and participating to a population-based cohort study (Progetto ATENA), underwent an ultrasound examination of the carotid arteries. Blood pressure, serum HDL-cholesterol, LDL-cholesterol, triglycerides, fasting glucose, insulin, apo B and hs-CRP were also measured in all participants.
Results. Women in the second and third tertile of body mass index showed an increased common carotid intima-media thickness compared with those in the first tertile: II vs I tertile (O.R.=1.78, p=0.056), III vs I tertile (O.R.=2.21, p=0.018), adjusted for age and metabolic syndrome. Obese and overweight p-m women showed increased common carotid lumen diameters as compared to lean p-m women (mean ± SEM: 6.04±0.06 mm; 5.90±0,06 mm and 5.68±0.06 mm respectively; p<0.01 [obese vs lean] and 0.046 [overweight vs lean]); no statistical difference was found between obese and overweight p-m women. The statistical significance between obese and lean p-m women was retained even after adding as covariates the components of the metabolic syndrome.
Conclusion. These findings indicate an association between general obesity and preclinical carotid artery abnormalities, independently of metabolic syndrome, in a population of p-m women.

GENETIC VARIANTS BESIDES CYP2C19*2 POLYMORPHISM ARE ASSOCIATED WITH MAJOR ADVERSE CARDIOVASCULAR EVENTS IN HIGH RISK VASCULAR PATIENTS ON DUAL ANTIPLATELET THERAPY

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Persistent platelet reactivity despite antiplatelet treatment confers an increased risk of major adverse cardiovascular events (MACE) in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) with stent implantation. Genetic and nongenetic mechanisms are involved in high on-treatment platelet reactivity. Recently, the CYP2C19*2 polymorphism has been demonstrated an independent determinant of MACE occurrence in patients on antiplatelet therapy. In addition to polymorphisms linked to clopidogrel metabolism, genetic variants in different genes coding for platelet receptors and enzymes have been investigated with discordant results. In 922 consecutive ACS patients undergoing PCI with stent implantation on dual antiplatelet therapy followed up for 6 months, aim of our study was to assess the relation to the risk of MACE of 41 allelic variants in 14 genes coding for prostaglandin-endoperoxide synthase-1 (PTGS1 or COX1), -2 (PTGS2 or COX2), von Willebrand factor (VWF), integrin alpha 2b (ITGA2B), glycoprotein Ib (GP1BA), glycoprotein VI (GP6), selectin P (SELP), integrin alpha 2 (ITGA2), fibrinogen beta chain (FGB), purinergic receptor P2Y12 (P2RY12), ATP-binding cassette sub-family B member 1 (ABCB1), different isoforms of CYP450 (-3A4, -2C9, and -2C19). Genotyping was performed with the specific allelic discrimination Taqman assays. We confirmed the independent association of the CYP2C19*2 polymorphism with the occurrence of MACE. Moreover, patients with MACE showed a higher prevalence of carriers of the rs3842788 COX1 polymorphism and of homozygotes for the rs7969672 VWF polymorphism, and a lower prevalence of carriers of the rs5911 ITGA2B polymorphism (18.5% vs 7.9%, p=0.007; 7.4% vs 2.3%; p = 0.022 and 46.3% vs 61.7%, p=0.023, respectively); at multivariate regression analysis only COX1 polymorphism remained significantly associated to MACE [OR=3.4 (1.45-8.02), p=0.005]. Our data indicated that, besides the well known CYP2C19*2, other polymorphisms in genes involved in platelet function play a crucial role in the outcome of high risk vascular patients.

ANGIO-MODULATION IN ENDOTHELIAL CELLS: AN UN-EXPECTED ROLE OF DESMOGLEIN-2 IN REGULATING ACTIN DYNAMICS AND ITS RELEVANCE TO ANGIOGENESIS DEREGULATION IN SYSTEMIC SCLEROSIS

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Previous observations underlined the anti-angiogenic properties of endothelial cells of patients affected by the diffuse form of Systemic Sclerosis (SSc) leading to microvessel sufferance, capillary loss and then organ failure. In two previous studies we demonstrated that microvascular endothelial cells from SSc patients (SSc-MVECs), which largely over-express pro-angiogenic factors, also over-produce anti-angiogenic molecules and are defective for systems required to perform a suitable angiogenic program.
Among the most striking difference between SSc-MVECs and normal MVECs (N-MVECs) was the down regulation of desmoglein-2 (DSG2). DSG2 function in normal and defective angiogenesis has not yet studied. Aim of our study was to investigate by siRNA-dependent loss of function, the role of DSG2 in N-MVECs. After silencing, DSG2 expression at mRNA level was reduced of about 14 fold. The amount of DSG2 protein upon the treatment is down-regulated to levels similar to those expressed by SSc-MVEC.
Both Matrigel invasion in Boyden chamber assay and capillary morphogenesis were impaired, with a phenotype similar to that previously reported for SS-MVEC subjected to the same assays. To investigate the biological processes and pathways of the MVECs altered by the reduced expression of DSG2, Affymetrix Gene Expression Profiling of N-MVECs and N-MVECs after siRNA silencing of DSG2 gene expression (siDSG2-N-MVECs) was performed, showing the differential expression of 2,945 genes. After functional classification of the 2,945 genes, we observed a high number of functional terms and pathways implied in angiogenesis, blood vessel development, cytoskeleton organization and biogenesis.
These observations were validated by molecular and functional evaluation of 7 genes (MACF1, DIAPH1, DIAPH2, ARPC3, RAC2, CDH5, and ITGB8) involved in cytoskeleton organization. These data together with those of both impaired Matrigel invasion and capillary morphogenesis suggested a crucial role of desmoglein-2 in regulating actin dynamics and its relevance to angiogenesis deregulation in SSc, an in vivo model of anti-angiogenesis.

DIFFERENTIAL GENE EXPRESSION PROFILES OF WHOLE PERIPHERAL BLOOD IDENTIFY THREE GROUPS OF ACUTE CORONARY SYNDROME PATIENTS UNDERGOING PCI

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The pathophysiological mechanisms underlying the acute coronary syndrome (ACS) in its multiple manifestations remain to be finely understood. Aim of our study was to evaluate the peripheral gene expression profile of ACS patients undergoing percutaneous coronary intervention (PCI) on dual antiplatelet therapy. We evaluated the gene expression profiles by Affymetrix technology (47,000 transcripts) on RNA extracted from whole peripheral blood of patients. By an unsupervised clustering analysis of 29 patients, we identified three different groups of patients (A, n=9; B, n=11; and C, n=9). The three groups were comparable for sex, and significantly differed for age [A=76(71-89); B=74(63-89); C=68(55-74)]. Group A, B and C showed respectively 66.7%, 27.3% and 33.3% of patients with ≥3 vessels with a stenosis >50%, and 55.6%, 9.1% and 44.4% of patients with ≥3 traditional cardiovascular risk factors. Interestingly, in group A we observed 88.9% of patients with hypertension compared with 45.4% in B and 44.4% in C; in group C 66.7% of patients were dyslipidemic with respect to 33.3% in A and 18.2% in C; in group B 90.9% of patients had residual platelet reactivity on antiplatelet treatment by both ADP- and arachidonic acid-induced platelet aggregability with respect to A (66.7%) and C (33.3%). The Significance Analysis of Microarrays identified 8464 differentially expressed genes between group A and B, 8945 between A and C, and 3223 between B and C. Interestingly, among genes with a different profile in the 3 groups there were genes coding components of the methionine metabolism (MTHFR, MTHFD2, MTR, MTRR, FOLR1, SLC19A1 DHFRL1, AHCYL1), of the TGF pathway (TGFB, TGFBR1, TGFBR2, SMAD1, SMAD2, SMAD4), and of the superfamily of the phospholipase A2 (PLA2G7/Lp-PLA2, PLA2G6/iPLA, PLA2G4A/cPLA2alpha, PLA2G12A/sPLA2, PLAA). Our data identify differential profiles underling different pathophysiological mechanisms in ACS patients and biological markers to consider in the diagnosis and prognosis.

PRESERVED HDL FUNCTION INDEPENDENT OF INFLAMMATION DURING ACUTE MYOCARDIAL INFARCTION

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Objective. The acute-phase inflammatory response (APR) is a protective reaction to various noxious stimuli, including an acute myocardial infarction (AMI). Aim of the study was to assess the

changes of HDL structure and function in patients with AMI of comparable severity but with an APR of different intensity.
Methods and Results. Patients were divided into those developing slight (Low-APR, n=11) or severe APR (High-APR, n=11) according to their maximum plasma CRP levels. Blood samples were collected at admission and at APR peak.
At admission, HDL from High-APR patients were enriched in LpA-I:A-II and depleted of PON-1 if compared to Low-APR HDL. At APR peak, a selective depletion of LpA-I:A-II particles and prebeta-HDL, an enrichment in SAA and an increase of HDL size were detected in High-APR HDL, while no changes were observed in Low-APR HDL.
The impact of these changes on HDL function was then assessed. No significant changes in sera and HDL ability to promote cell cholesterol efflux through ABCA1, ABCG1 and SR-BI were detected between admission and APR peak in both groups of patients. At APR peak, HDL from both groups retained their ability to inhibit inflammation and to increase eNOS protein levels in endothelial cells. HDL-induced eNOS activation was also comparable between admission and APR peak in both groups, but High-APR HDL were less effective than Low-APR ones at both time-points.
Conclusions. During an AMI, HDL retained their ability to promote cell cholesterol efflux and to maintain endothelial cell homeostasis independent of inflammation-induced structural changes, thus indicating that in this pathologic condition HDL can not be defined as "dysfunctional" particles.

RHEOLOGICAL PARAMETERS IN ELDERLY: RESULTS FROM THE INCHIANTI STUDY

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Advancing age is an important risk factor for cardiovascular disease among men and women. Hyperviscosity iscaused by alterations of blood cells, mainly red blood cells, and plasma components (mainly fibrinogen) and it is associated with increased risk of cardiovascular diseases.Several factors are able to influence whole blood and plasma viscosity. Scarce data are available about the relation between plasma,whole blood viscosity and advanced age.
We used data from the 6-year follow-up of the InChianti Study (n=948) to determinethe relation between advanced age and blood rheology parameters. In the InChianti Study whole blood viscosity (WBV)was measured at 37°C by using a Rotational Viscosimeter at share rate of 0.512 second-1, plasma viscosity (PLV) at shear rate of 20.40 second-1. Whole blood viscosity values adjusted for a haematocrit of 44% inmen and of 40% in women aretermed HCT-corrected WBV. WBV and HCT-corrected WBV were significantly related with age, body mass index, lipid parameters, systolic and diastolic blood pressure, physical activity levels and cigarette pack-years.
PLV values significantly correlated with fibrinogen levels, but no correlation with age was observed. WBV and hematocrit values were lower in the highest age quartile with respect to lowest quartile, whereas HCT-corrected WBV were higher in the highest age quartile with respect to lower quartiles.
General linear model adjusted for sex, cardiovascular risk factors and lipid parameters demonstrated that age is a predictor of WBV and hematocrit. In conclusion, our study provide further insight into the complex interaction between rheologic parameters, cardiovascular risk factors and age, suggesting that advanced age, in addition to several cardiovascular risk factor, is a significant determinant of WBV.

FIBRIN RESISTANCE TO LYSIS IN CORONARY ARTERY DISEASE PATIENTS AND PLATELET HYPER-REACTIVITY IN CORONARY ARTERY DISEASE PATIENTS

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Altered properties of the plasma clot architecture, as evidenced by decreased clot permeability, are demonstrated in patients with coronary artery disease (CAD) who underwent to percutaneous coronary intervention (PCI), but scarce information is available on fibrin resistance to lysis in CAD. Platelet hyperreactivity in patients on dual antiplatelet treatment represents a risk factor for the occurrence of adverse cardiovascular events.
Aim of the present study was to ascertain whether fibrin resistance to lysis occurs in CADpatients on dual antiplatelet therapy and its relationship with platelet hyper-reactivity. We studied 57 CAD patients (18F/39M) on dual antiplatelet therapy6 months after PCI and 33 controls (10F/23M) of equivalent age.Fibrinogen was purified from citrated plasma and exposed to thrombin. Plasmin-mediated cleavage of fibrin beta chain was assessed hourly over a 6-hour period by polyacrylamide gel electrophoresis and fibrin band intensity was measured by densitometry of stained gels. Residual platelet reactivity (RPR-aggregation by collagen≥56%.) was assessed in platelet-rich plasma stimulated by 2 microg/ml collagen. After 6 hours in all controls degradation of the fibrin beta chain occurred, whereas it was not observed in 27 (47.6%) CAD patients. A significant decline in fibrin band intensity was observedin 29 (50.9%) CAD patients.
Degradation of the fibrin beta chain was not significantly different between patients STEMI(14/28, 50%) and non-STEMI (15/29, 51.8%) patients and between patients with and without traditional risk factors. The decline in fibrin band intensity was significantly ($p<0.05$) different between patients with and without RPR by collagen:the degradationof fibrin did not occur in 14/20 (70%) patients with RPR by collagen and in 15/37 (39.4%) patients without.
Persistence of fibrin beta chainoccurs in CAD patients and is related to platelet hyper-reactivity, suggesting a new pathophysiological mechanism underlying thrombus formation.

LIPOPROTEIN(A) AND FAMILY HISTORY OF CARDIOVASCULAR DISEASE IN CHILDREN WITH FAMILIAL DYSLIPIDEMIAS

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Objective. Family history of cardiovascular disease (CVD) and increased lipoprotein(a) [Lp(a)] levels represent emergent CVD risk factors. Dyslipidemia occurring since childhood strongly

contributes to increase the CVD risk. We investigated in dyslipidemic children the impact of Lp(a) and lipid profile on CVD family history. Study design.
Lipid profile, Lp(a) levels and a two generation genealogical tree to detect cardiovascular events among 240 children with familial dyslipidemias were evaluated. Lp(a) levels were stratified according to presence, age of occurrence, number and type of cardiovascular events in their kindreds.
Results. We did not observe differences in Lp(a) and other plasma lipid fractions between children with or without family history of cardiovascular events. However, the percentage of children with elevated Lp(a) (≥85th percentile) was higher among those with a positive family history for early cardiovascular events (p=0.01). Lp(a) was a significant independent predictor of the number of premature cardiovascular events (β=0.18, p=0.01) and of cerebrovascular events in the kindreds (OR 2.5, 95% CI: 1.06-6.05, p=0.040), independent of plasma lipid fractions and other cardiovascular risk factors.
Conclusions. In children with familial dyslipidemias the overall association between Lp(a) and family history of precocious CVD is due to a threshold effect among children showing the highest Lp(a) levels.
However, multiple cardiovascular and cerebrovascular events are significantly predicted by any Lp(a) plasma level increase, independently of other cardiovascular risk factors.

ELEVATED C-REACTIVE-PROTEIN AND CAROTID ATHEROSCLEROSIS IN CHILDREN WITH SLEEP-BREATHING DISORDERS

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Background. Sleep-breathing disorders (SBD) in children are characterized by prolonged partial upper airway obstruction and/or intermittent complete obstruction that disrupt normal ventilation during sleep. Main risk factors include adenotonsillar hypertrophy, obesity, and neuromuscular disorders. Several large-scale, prospective, epidemiologic studies have shown that plasma levels of High-sensitivity C-Reactive Protein (Hs-CRP) in adults are a strong independent predictor of risk for cardiovascular morbidity.
Moreover, CRP levels are associated with overweight and obesity. Carotid intima-media thickness (IMT) is a well accepted marker of atherosclerosis both in adults and children and increased thickening is associated with several cardiovascular risk factors.. Aim of our study was to test the influence of SBD on CRP levels and carotid IMT in a group of healthy children, both lean and obese, not selected for primary snoring.
Patients and Methods. 150 children without other co-morbidities (age 5-15 yrs) attending the Outpatient Weight Clinic of the Department of Pediatrics, A. Cardarelli Hospital (Naples, Italy) or scheduled for a standard routine visit by their ambulatory paediatrician were asked and 130 accepted to participate in this study. After exclusion criteria were applied, 101 children remained and constituted the present cohort. Anthropometric measures and biochemical tests were performed in all children. Quantitative B-mode ultrasound scans were used to measure intima-media thickness (IMT) of the common carotid artery. Polysomnographic studies were performed overnight in a sleep laboratory in all children. AHI (Apnea-Hypopnea Index) was the number of hypopneas, obstructive and mixed apneas per hour of sleep.
Results. In the whole group of 101 children AHI was significantly associated with Hs-CRP concentrations (r=0.32, p = 0.002) and this association was maintained adding age and sex as covariates. Obese children had 3.3 times more probability to have SBD (HR 3.3, 95% CI 1.2 - 9.3, p=0.02) than the reference group of non-obese children. Dividing the whole cohort of children in two groups, the first group with SBD (AHI ≥5) and the second without SBD (AHI <5) we found borderline significant differences in BMI (p=0.05). In the group of obese children with SBD there was a statistically significant difference in Hs-CRP values as compared to obese children without SBD, after adjustment for age, sex and ponderal excess (p=0.03).
Conclusions. Main findings of the present study were: an association between SBD and CRP concentrations, mainly mediated by overweight and obesity; a low-grade inflammation in obese children with SBD, significantly greater than in obese children without SBD; lack of significant association between SBD and subclinical atherosclerosis in children.

ASSOCIATIONS BETWEEN CIGARETTE SMOKING AND SERUM LIPID LEVELS IN AN ITALIAN COHORT: THE BRISIGHELLA HEART STUDY

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Background and Aims. Cardiovascular disease is the leading cause of mortality and morbidity in the developed countries, and is promoted by different risk factors including cigarette smoking and modification of blood lipid profile too. Purpose of this study is to investigate how cigarette smoking influences blood lipids profile.
Materials and Methods. Brisighella Heart Study is an observational longitudinal study with a 4-year follow-up aiming to evaluate the spontaneous trend of major cardiovascular risk factors in a free living Italian population of the Emilia Romagna. In this work we analyzed data belonging to persons who underwent checks both in 1988 and 1996 (male: 727; female: 795), that were divided by smoking habits (smokers vs non-smokers), sex and age (adult people, 30-64 yr, vs old people, > 64 yr); female population was also divided by the presence of menopause. More, using the cut off of metabolic syndrome as defined by ATP III, we valuated the different risk to develop low HDL-C levels between smokers and non-smokers.
Results. We analysed the spontaneous trend of the mean lipid parameters (TC, LDL-C, HDL-C, TG), finding a statistically significant decrease of HDL-C levels both smokers and non-smoker, but smokers have significant lower HDL C levels, both 1988 (53.8 vs 57.4 mg/dL, p<0.001) and 1996 (50.2 vs 53.3 mg/dL, p<0.001). After adjusting for sex, age and the presence of menopause smokers keep on lower HDL C levels only in adult men (1988: 49.5 vs 52.7 mg/dL, p<0.05; 1996: 45.7 vs 49.3 mg/dL, p<0.01) and postmenopausal women (1988: 54.9 vs 60.2 mg/dL, p<0.05; 1996: 52.0 vs 55.6 mg/dL, p<0.05). Using the cut off of metabolic syndrome as defined by ATP III, we found a higher risk to develop low HDL-C levels for smoker men (HR: 1.40, 95% CI: 1.00 - 1.92, p<0.05) and postmenopausal women (HR: 1.50,

95% CI: 1.08 - 2.08, p<0.05). We didn't find significant data about the influence on the other main lipid parameters.
Conclusion. This study suggests that smoking habit influences HDL-C level in all men, especially adults, and in menopausal female population.

ASYMMETRIC DIMETHYLARGININE DOES NOT CONTRIBUTE TO ENDOTHELIAL DYSFUNCTION IN SUBJECTS WITH ABNORMALITIES OF GLUCOSE REGULATION

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Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase (eNOS), has been associated with endothelial dysfunction and atherosclerosis. Increased plasma levels of ADMA have been described in diabetic subjects with nephropathy or cardiovascular disease. Studies assessing ADMA levels in people with uncomplicated type 1 or type 2 diabetes report conflicting results. Materials and methods: Circulating levels of ADMA, SDMA (symmetrical dimethylarginine) and L-arginine (L-arg) together with brachial artery endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent dilation by 25 μg sublingual glyceryl trinitrate (GTN) were evaluated in 26 subjects with normal glucose tolerance (NGT), 34 with pre-diabetes (IFG or IGT, pre-DM) and 18 newly diagnosed type 2 diabetics (newT2DM) identified through OGTT. Plasma concentrations of ADMA, SDMA and L-arg were determined simultaneously by high-performance liquid chromatography. FMD and GTN were assessed by high-resolution ultrasound and computerized edge detection system. Groups showed similar distribution for gender, smoking habits, BMI, waist circumference,dBP, total and LDL cholesterol, apoA1 and apoB, fibrinogen, and fasting insulin. Namely, there were no differences in eGFR and cystatin C. Age (55±5, 53±8 vs 48±9 years, p=0.01), fasting and post-load OGTT glucose, glucose area under the OGTT curve (AUCgluc), HbA1c (6.0±0.4, 6.5±0.6 vs 5.5±0.4%, p<0.0001) and triglycerides were higher in pre-DM and newT2DM than in NGT; HDL cholesterol was lower. In pre-DM, sBP (127±13) was in between NGT (118±15) and newT2DM (137±15 mmHg, p=0.0005). GTN decreased (D% 9.9±3.4, 8.8±3.3 and 7.4±3.9, m±sd; p=0.081) with significant differences between NGT and newT2DM (p=0.025). FMD was lower in newT2DM (D% 4.4±3.3, m±sd) and in pre-DM (D% 6.0±2.8) compared with NGT (D% 7.9±3.6, p=0.0017). L-arg levels were similar in NGT (97.5±20.0) and pre-DM (97.1±20.6), lower in newT2DM (81.2±18.9 μmol/l, p=0.015). ADMA progressively reduced from NGT (1.33±0.96 μmol/l) to pre-DM (1.02±0.79 μmol/l, p=0.14 vs NGT) and newT2DM (0.72±0.53 μmol/l, p=0.017 vs NGT;ANOVA, p=0.05). SDMA was similar in NGT (1.78±0.74) and pre-DM (1.56±1.02, p=0.31),reduced in newT2DM (0.97±0.35 μmol/l, p=0.002 vs NGT, p=0.02 vs pre-DM; ANOVA, p=0.006). No association was observed between ADMA (or SDMA) and eGFR or cystatin C. No correlation emerged between ADMA and FMD (r=0.14, p=0.23) with a weak one between ADMA e GTN(r=0.29, p=0.014). By multiple regression, AUCgluc (p=0.002) and sBP (p=0.047), but not ADMA were inversely related with FMD. AUCgluc, inversely, (p=0.024) and ADMA (0.044) correlated with Conclusion: We suggest that uncomplicated newT2DM and subjects with pre-DM have lower circulating ADMA than nondiabetic control subjects, in presence of impaired endothelium-dependent flow-mediated dilation. ADMA levels are not related to endothelial function. In these subjects with early abnormalities of glucose regulation, endothelial dysfunction seems not a result of eNOS inhibition by ADMA.

EFFECTS OF N-3 PUFAS THERAPY ON LIPID PROFILE, INFLAMMATORY BIOMARKERS, AND MARKERS OF VASCULAR REMODELLING IN A BASELINE CONDITION, AND AFTER AN OFL IN DYSLIPIDEMIC PATIENTS

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Aim. With this study we wanted to evaluate how a 6 months treatment with ω-3 polyunsaturated fatty acids (n-3 PUFAs) can modify lipid profile, inflammatory biomarkers, and markers of vascular remodelling in a baseline condition, and after an OFL in patients affected by combined dyslipidemia.
Materials and Methods. A total of one hundred and sixty-seven patients affected by combined dyslipidemia were enrolled in the study; patients were assigned to receive, as addition to diet and physical activity, placebo or n-3 PUFAs 1 g three times a day, during the meals, for 6 months. Body mass index (BMI), body weight, fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), blood pressure, lipid profile, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), interleukin-6 (IL-6), high-sensitivity C reactive protein (Hs-CRP), soluble E-selectin (sE-selectin), tumor necrosis factor-a (TNF-a), metalloproteinase-2 (MMP-2), and metalloproteinase-9 (MMP-9) were evaluated at the baseline, and after 2, 4 and 6 months.
Results. In a baseline condition, we observed a better improvement of lipid profile, and of all inflammatory, and vascular remodelling parameters considered with n-3 PUFAs compared to placebo. On the other side, comparing the OFL performed at the baseline and after 6 months of treatment, there was an improvement of all parameters peaks after the OFL performed at the end of the study in the group treated with n-3 PUFAs, but not in the one treated with placebo.
Conclusions. N-3 PUFAs were better than placebo in giving an improvement of lipid profile, and of all inflammatory, and vascular remodelling biomarkers, both in a baseline condition and after an OFL.

TOYOTISM MODELING INSIDE THE REGIONAL NETWORK FOR INHERITED LIPID DISORDERS

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It is a general trend of today's hospital management to shift towards toyotism, id est towards managerial modeling of clinical and organizational pathways based on Toyota derived principles. KAI ZEN which is the key concept of toyotism means to de construct a built setting of care (KAI) in order to construct a new better establishment (ZEN). This transformation may help a continuous flow of add-value all over along the step. We analyzed toyotism modeling and application inside the coordination center of the network by organizing a Kaizen work shop (short intensive one week work) in order to assesses the possibility of obtaining a minimal core of toyotism in ambulatory care based on the following toyotism key principles • Order and politeness (5S) • Autonomous handling of path • Takt time obtaining (a good flow of stream value) We assumed a bounded rationality decision making models as compliant with toyotism (Simon and Nonaka) . Principal step outlined were • Identification of steps without add-value (do step in order identify the burden of waste) • vascular and lipid facilities integration in a common path with an identified process manager (plan step in order to identify improvements and responsibilities) • amelioration of diagnostic tools perception and tacit knowledge (Check step) Our stand point is that toyotism shifts the focus from standard performing to flow performing in order to grow up organizational tacit knowledge. Kaizen work shop is a meaningful human situation which may be extended from coordination center to external network centers. Takt time obtaining may be supported from rapid action and strong commitment.

ATP-BINDING CASSETTE TRANSPORTER A1 (ABCA1) OVEREXPRESSION IN HYPERCHOLESTEROLEMIC PLAQUES:A POTENTIAL MOLECULAR EXPLANATION

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Background. Atherosclerosis is a progressive inflammatory disease representing the first cause of mortality worldwide. Increasing attention is being given to the role of ATP-binding cassette transporter A1 (ABCA1) in the development of atherosclerotic plaques. ABCA1 is a trans-membrane protein involved in reverse cholesterol transport and HDL formation. Its importance in cholesterol metabolism and atherosclerosis is confirmed by the increased grade of atherosclerosis in patients affected by Tangier disease (ABCA1 deficiency). Interestingly, ABCA1 may contribute to atherosclerosis development not only by influencing reverse cholesterol transport but also by a direct effect on the vessel wall.
Previous findings suggest that ABCA1 mRNA is more expressed in advanced atherosclerotic lesions than in normal arteries and early atheromatous changes. In particular, it has been demonstrated that ABCA1 is abundantly expressed in cholesterol-loaded macrophages, and that a major mechanism of regulation of the ABCA1 gene is the intracellular level of cholesterol and its bioproducts oxysterols.
However, at this time the molecular mechanism(s) linking cholesterol dysmetabolism and ABCA1 upregulation is still largely unknown. MicroRNAs (miRNAs) are short, endogenous, noncoding, single-stranded RNAs molecules and represent an emerging class of gene regulators. Growing evidences suggest their implication in cardiovascular pathology. In the first phase of this project, using complex bioinformatic tools for target gene prediction (miRBase) we have recently estimated that miR-145 may deeply regulate ABCA1 expression. Thus, the aim of this final study was to investigate the expression level of ABCA1 and miR-145 in human atherosclerotic plaques, and to correlate their expression with the presence of hypercholesterolemia.
Material and Methods. Thirty-seven human atherosclerotic plaques were selected from the MediCA (Mediterranean Carotid Atherectomy) biobank, a series of 450 atherosclerotic plaques obtained from patients who underwent elective carotid endarterectomy for high-grade (>70%) vessel stenosis. Twenty-six of these 37 plaques were from patients carrying hypercholesterolemia as the only conventional cardiovascular risk factor (hypercholesterolemic group), whereas 11 plaques were from non-hypercholesterolemic patients without any other main risk factor (control group).
The plaques were excised, appropriately dissected, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent molecular analyses. Total RNA and miRNAs were carefully extracted and transcribed into cDNA. Then, expression levels of the ABCA1 gene and miRNA 145 were assessed by real-time PCR with the 2-DDCT method.
Results. Atherosclerotic plaques from patients with hypercholesterolemia showed a statistically significant (p=0,037) overexpression of the ABCA1 mRNA when compared to plaques from control patients without hypercholesterolemia. Furthermore, a trend supporting an inverse correlation between ABCA1 mRNA and miRNA145 expression was also observed.
Conclusions. To the best of our knowledge, this is the first report suggesting that ABCA1 mRNA is significantly more expressed in the atherosclerotic plaques of patients with hypercholesterolemia.
This might reflect a local response to the abnormal accumulation of oxysterol in human plaques as consequence of hypercholesterolemia. The downregulation of miRNA 145 could represent the ultimate molecular mechanism linking oxysterol accumulation and ABCA1 gene expression.

CIRCULATING PROGENITOR CELLS AND HIGH DENSITY LIPOPROTEIN LEVELS IN ELDERLY

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Introduction. Circulating progenitor cells(CPCs), including early and late outgrowth endothelial progenitor cells (EPC), contribute to formation of new blood vessels, neovascularization and homeostasis of the vasculature. It was well established that senescence leads to a progressive reduction in the number of CPC, due both to increased turn-over, both to progressive depletion of bone marrow.
High-density lipoprotein-cholesterol (HDL-C) is a continuous inverse cardiovascular risk factor. HDL was also involved in decreasing inflammation, in preventing low-density lipoprotein oxidation, vascular endothelial cell apoptosis and thrombosis, and in improving vascular endothelial function. We followed up for five years (from 2004 up to 2009) a population of Sicilian elderly, in order to observe mortality, morbidity and survival. This study is focused on plasma lipid profile, particularly HDL-C levels, and CPC number in peripheral blood.
Patients and Methods. We included one hundred elderly outpatients (mean age 82.2±2.7 years), equally divided between males and females (52 m and 48 f); each subject was included after a careful medical history and clinical evaluation, particularly with regard to major cardiovascular risk factors (hypertension, diabetes mellitus, hypercholesterolemia, smoking); the biometric indices were measured. A blood sample was drawn at enrolment to evaluate clinical chemistry parameters and CPCs. During the observation period was recorded the death of forty eight subjects.
Results. The mean count of CPCs in elderly subjects was 3.0 ±1.4 cells/μL. Mean values of HDL-C were 62.6±10.9 mg/dl; both males and females had meanly HDL-C plasma concentrations considered as normal according to the ATP III classification (only six out of one hundred subjects presented plasma levels below 50 mg/dl). Dividing subjects into two groups, living and deceased after five years, we found that CPCs at enrolment were significantly higher in the group of long-term survivors (4.1±0.7 vs 1.9±1.0, p<0.001).
However, no difference was found in HDL-C plasma levels between the two group, even considering only the subjects died from cardiovascular causes (p=ns). Subjects with low cell counts have died on average 3.25 years for all causes. The multiple regression analysis indicated CPC number as the main independent factor able to affect the survival, although after the advanced age.
Conclusions. HDL-C is considered a continuous inverse cardiovascular risk factor. Low plasma levels, specially below 40 mg/dl and less, are an independent risk for cardiovascular disease, due to early atherosclerosis.
More recently, CPCs have been showed a main predictor of cardiovascular disease, and of all-causes mortality. Smoking, hypercholesterolemia and hypertension appeared to not affect significantly overall survival, except when associated with diabetes. Based on our data, we may conclude that the survival of elderly subjects is little affected by classical cardiovascular risk factors. On the other hand, in this population a low CPC number may mark a low survival. Further studies are needed to investigate the prognostic value of this important new marker.

LOW PLATELET COUNT IN PATIENT WITH CHRONIC ATRIAL FIBRILLATION

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Introduction. Literature reports conflicting data about platelet count in the acute and chronic phase of atrial fibrillation (AF). Nevertheless few works remak the presence of a low platelet in patients with chronic AF. We aimed to verify the presence of a low platelet count in patients with chronic AF and count changes in a cardiac surgery setting.
Materials and Methods. Out of the 1743 patients admitted to cardiac rehabilitation following cardiac surgery from January 2008 to May 2010 we included the 460 patients who underwent mitral valve plasty. The 76 patients with post-operative haemodinamics and infective complications where excluded. We analyzed the pre-operative and post-operative platelet (PLT) and white blood cell (WBC) counts of the patients in AF (n=103) and in sinus rhythm (SR) (n=281). All the results are expressed as median, interquartile and range. Cells count are expressed as number x 109 per liter.
Results. We found a pre-operative lower platelet count in patients with chronic AF than in patients in SR (168, 153-186 vs 217, 186,5-247; p<0.0021). Conversely, no difference was seen in the post operative PLT count both in the day of admission (AF 218, 185-272 vs SR 210, 182-244; p=ns) and in the day of final discharge (AF 252, 201,317 vs SR 290, 259-362; p=ns) from rehabilitation. No difference was found in the pre-operative WBC count (p>0.05) between patient in AF and in SN (5.8, 5.05-6.9 vs 6.6, 5.35-7.28).
Conclusion. Patients with chronic AF showed a lower platelet count not associated with a lower white blood cell count. The platelet counts of patient with chronic AF and in sinus rhythm, eventually, became similar after cardiac surgery for mitral valve repair. This is probably related to the response elicited by the surgical cardio-pulmonary bypass.

RELATIONSHIP BETWEEN VISCERAL ADIPOSITY AND CHOLESTEROL SYNTHESIS IN DYSLIPIDEMIC PATIENTS

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Rationale and Aim of the Study. Human blood contains small amounts of non-cholesterol sterols. Their quantification allows the study of cholesterol absorption and synthesis: plants sitosterol and campesterol are considered a surrogate index for cholesterol absorption, while lathosterol, a precursors of cholesterol, is considered a marker of synthesis. Previous findings suggested that different hyperlipemias have different patterns of cholesterol absorption (higher in polygenic hypercholesterolemia) and synthesis (higher in familial combined hyperlipemia); furthermore a pattern of low cholesterol absorption/high

synthesis was demonstrated in patients with obesity/insulin resistance/diabetes. In order to look insight the clustering of cholesterol synthesis markers with clinical and laboratory markers of obesity/insulin resistance, the purpose of our study was to investigate in hyperlipemic patients the relation of non-cholesterol sterols (campesterol, sitosterol and lathosterol), surrogate markers of absorption and synthesis, with visceral and subcutaneous fat, studied by means of ultrasonography.

Patients and Methods. We studied 126 patients referring to our Lipid Clinic with primary hyperlipemias (polygenic hypercholesterolemia, familial hypercholesterolemia, familial combined hyperlipemia, hypertriglyceridemia).

All subjects followed an isocaloric hypolipidic diet with extra virgin olive oil as the principal fat of the diet and treatments with hypolipemic drugs, fibers, phytosterols or soya were an exclusion criteria. In all participants plasma sitosterol, campesterol and lathosterol were determined by gas chromatography coupled to mass spectrometry; we also determined total cholesterol, triglycerides, HDL cholesterol, glycemia, insulinemia, apoprotein AI, apoprotein B.To correct for the effect of plasma lipid levels, non-cholesterol sterol concentrations were adjusted for plasma cholesterol (102 µmol/mmol cholesterol). Visceral fat area (VFA) and subcutaneous fat area (SFA) were evaluated by means of abdominal ultrasonosgraphy (MyLab 50, Esaote, Italy). We divided the study population on the basis of VFA median values in two groups, below /equal or above 154 cm^2.

Results. Patients with VFA above 154 cm^2 had significantly higher values of lathosterol (median 109 vs 76 102 µmol/mmol cholesterol p<.004), and of body mass index (p<.001), waist circumference (p<.001), blood pressure (p<.005), triglycerides (p<.05), insulin (p<.001), HOMA-IR (p<.001) and lower values of HDL-C (p<.001), apo AI (p<.001).In the same group sitosterol and campesterol showed significantly lower median values (77 vs 103 102 µmol/mmol cholesterol, p .005 and 31 vs 53 ±56, p.024 respectively).

Spearman's rank correlations showed a significant positive correlation between visceral fat area and lathosterol ("ρ" .35, p<.001); a negative correlation was observed between VFA and HDL-C ("ρ"-.43, p<.001), apoprotein AI ("ρ"-.49, p<.001), campesterol ("ρ" -.23, p.01), and sitosterol ("ρ"-.35, p<.001). Subcutaneous fat area did not show any correlation with non-cholesterol sterols. Stepwise regression showed VFA as an independent predictor of lathosterol values (β .389, p<.0001, p of the model <.0001); age, systolic blood pressure, BMI, waist circumference, triglycerides, HDL-C and HOMA were also inserted in the models but failed to enter the final equation.

Conclusions. In this group of hyperlipemic patients the bulk of visceral fat conditions the amount of cholesterol synthesis.The use of ultrasonography for the detection of abdominal adiposity discriminated the impact of the two fat components on cholesterol metabolism, allowing a better characterization of cholesterol pathway, potentially useful for a tailored therapeutic approach.

THE HELSINKI FIELD SUBSTUDY: EFFECTS OF FENOFIBRATE AND HOMOCYSTEINE ON IN VITRO CHOLESTEROL EFFLUX POTENTIAL OF HIGH DENSITY LIPOPROTEIN (HDL) AND PLASMA

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Introduction. HDL has several antiatherogenic functions such as role in reverse cholesterol transport (RCT), antioxidant, anti-inflammatory, vasodilating and antithrombotic properties. Drug design during recent years has shown targeted interest to HDL metabolism. In the Field fenofibrate arm, HDL-C and apoA-I changes correlated inversely to changes in homocysteine (Hcy) raising the question whether Hcy counteracts fenofibrate effect on cardiovascular outcomes. Hcy-thiolactone, a metabolite of Hcy, can modify HDL apolipoproteins impairing their functions. A key atheroprotective mechanism of HDL is thought to be its role in RCT and therefore we investigated whether fenofibrate or Hcy modulate in vitro cholesterol efflux to HDL and plasma.

Methods. We selected 33 subjects in the fenofibrate arm according to quartiles of Hcy at 5th year: 17 subjects were in the lowest (low Hcy group) and 16 subjects were in the highest quartile (High Hcy group). In addition, 14 subjects (control group), allocated to placebo, were matched by Hcy levels to low Hcy group. This design allowed us to examine the effects of fenofibrate per se (comparison between control versus low Hcy groups at 5th year) and the effect of Hcy (comparison between low and high Hcy groups at 5th year) on HDL and plasma potential to function as acceptors in the cholesterol efflux process. Cholesterol efflux from labeled acetyl-LDL-loaded human THP-1macrophage-foam cells to individual HDL (15 µg protein) and plasma (0.5%) from the study subjects at baseline and at 5th year were measured for low and high Hcy groups; in the control group only 5th year samples were examined. The results are expressed as percentage (%) cholesterol efflux to acceptors.

Results. At the 5th year cholesterol efflux to HDL and plasma were similar between control (mean ±SD efflux to plasma and HDL: 16±4.4; 9.6±2.7) and Low Hcy groups (mean efflux to plasma and HDL 17.4±7.1; 7.8±3.2), both having similar levels of Hcy (mean 13.3±0.7 vs 13.2±2 µmol/L, respectively). The cholesterol efflux to plasma and HDL at baseline and at the 5th year were comparable in both low (baseline mean ± SD efflux to plasma and HDL 13.1±3.4; 5.9± 1.7) and high Hcy fenofibrate groups (baseline mean ± SD efflux to plasma and HDL: 16.9±4; 8.4±3.1; 5th year mean ±SD efflux to plasma and HDL: 17.7±5.1; 9.1±3.1), despite different levels of Hcy at the 5th year (mean 13.2±2 in low Hcy vs 27.4 ±6.5 µmol/L in high Hcy).

Conclusions. Fenofibrate treatment and high Hcy levels have no effect on either HDL or plasma potential to remove cholesterol from foam cells. However, there are other atheroprotective functions associated with HDL beyond its role in RCT and Hcy can attenuate these processes.

GENETIC VARIANTS WITHIN PNPLA2 INFLUENCE LIPID EXPRESSION IN FAMILIAL COMBINED HYPERLIPIDEMIA

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Objective. Familial combined hyperlipidemia (FCHL) has been associated to abnormalities in fatty acid metabolism. The adipose triglyceride lipase (PNPLA2) plays a pivotal role in the turnover of fatty acids in adipose tissue and liver. This study was designed to evaluate whether selected PNPLA2 variants may influence the susceptibility to FCHL or its lipid related traits.
Methods. Four SNPs within the PNPLA2 gene (rs7925131, rs7942159, rs66460720 and the nonsynonymous P481L) were selected based on previous association with decreased plasma levels of free fatty acids (FFA) and total triglycerides (TG) and their high frequency (MAF>0.25). These SNPs were genotyped in 214 FCHL individuals from 83 families and in 103 controls and the corresponding haplotypes were reconstructed.
Results. No association between individual SNPs and the FCHL trait was observed. However, two PNPLA2 haplotypes were associated to lower risk of FCHL ($P<0.004$ after Bonferroni's correction). Compared to the others, these haplotypes were related to lower TG (118.9 ± 66.8 vs. 197.1 ± 114.7 mg/dl; $P=0.001$) and higher HDL-C (62.3 ± 15.8 vs. 51.0 ± 15.0 mg/dl; $P<0.005$). In a subgroup of studied subjects (n=63) protective haplotypes were also associated to lower FFA levels (0.33 ± 0.11 vs. 0.46 ± 0.18 mEq/L; $P<0.05$). These effects were independent from age, BMI and HOMAIR.
Conclusion. These data demonstrate that variants within PNPLA2 may modulate the TG component of FCHL trait, thus implicating PNPLA2 as modifier gene in this lipid disorder. They also suggest a potential role of PNPLA2 in the metabolism of TG-rich lipoproteins.

ACTIVATION OF THE LIVER X RECEPTOR PROTECTS FROM DIABETES-INDUCED PERIPHERAL NEUROPATHY

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Neuroactive steroids act in the peripheral nervous system as physiological regulators and as protective agents for acquired or inherited peripheral neuropathy.In recent years, modulation of neuroactive steroids levels has been studied as a potential therapeutic approach to protect peripheral nerves from damage induced by diabetes. Nuclear receptors of the LXR family regulate adrenal steroidogenesis via their ability to control cholesterol homeostasis. Here we show that rat sciatic nerve expresses both LRXα and β isoforms and that these receptors are functional. Activation of Liver X Receptors using a synthetic ligand results in increased levels of neurosteroids and protection of the sciatic nerve from neuropathy induced by diabetes. LXR ligand treatment of streptozotocin-treated rats increases expression in the sciatic nerve of steroidogenic acute regulatory protein (a molecule involved in the transfer of cholesterol into mitochondria), of the enzyme P450scc (responsible for conversion of cholesterol into pregnenolone), of 5α-reductase (an enzyme involved in the generation of neuroactive steroids) and of classical LXR targets involved in cholesterol efflux, such as ABCA1 and ABCG1. These effects were associated with increased levels of neuroactive steroids (e.g., pregnenolone, progesterone, dihydroprogesterone and 3α-diol) in the sciatic nerve, and with neuroprotective effects on thermal nociceptive activity, nerve conduction velocity, and Na+, K+-ATPase activity. These results suggest that LXR activation may represent a new pharmacological avenue to increase local neuroactive steroid levels that exert neuroprotective effects in diabetic neuropathy.

PHARMACOLOGICAL TREATMENT OF A SARDINIAN PATIENT AFFECTED BY AUTOSOMAL RECESSIVE HYPERCHOLESTEROLEMIA

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Introduction. Autosomal recessive hypercholesterolemia (ARH) is a rare disorder caused by mutations in a gene encoding for a putative adaptor protein ARH required for receptor-mediated uptake of LDL particles in polarized cells, like hepatocytes. The disease presents a clinical phenotype similar to that of classical homozygous familial hypercholesterolemia (FH) caused by defects in the LDL receptor gene but is more variable, generally less severe, and more responsive to lipid-lowering therapy than homozygous FH.
Patients and Methods. An ARH 45 years old Sardinian patient (female) who refused plasmapheresis, the elective ARH therapy, was treated in our Lipid Centre with the association ezetimibe 10 mg plus rosuvastatin 40 mg per day. Lipid parameters were analyzed according standard procedures. The mean lipid values before and during treatment are reported in the table:

Serum parameters	No therapy	Ezet.10+Rosu.40
Total cholesterol	656	231
HDL-cholesterol	51	59
LDL-cholesterol	591	160
Triglycerides	71	62
Apoprotein-AI	124	133
Apoprotein-B	235	131
Lp(a)	14,0	15,7
CRP	3,4	2,8

Plasma liver enzymes underwent a small increase during treatment, but remained in the normal range.
Conclusion. Our results show a striking decrease ofserum lipid parameters during a 5-month treatment with ezetimibe 10 mg/rosuvastatin 40 mg per day. Pharmacological therapy was well tolerated without side effects. Xanthomas were reduced in size. Since the patient was found to carry some genetic variants

(homozygosity for c.-64 C>T and p.L21 dupl in PCSK9 gene and heterozygosity for c.816 C>G in NPC1L1 gene), which we have previously found to improve the response to statins and to ezetimibe, respectively, we suggest a possible genetic contribution to good response to the lipid lowering treatment in this patient and conclude that the combination ezetimibe/rosuvastatin can be an alternative therapy to the plasmapheresis in some ARH patients.

BETAINE DOWNREGULATES TISSUE FACTOR IN ACTIVATED HUMAN ENDOTHELIAL CELLS: A NEW PATHWAY OF VASCULAR PROTECTION

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Background. Numerous pieces of evidence suggest that moderately elevated levels of homocysteine are associated with arterial, venous thrombosis and atherosclerosis. Atherosclerosis is now recognized as a subacute inflammatory condition of the vessel wall where the presence of tissue factor (TF) is considered to be responsible for the major complication of atherosclerosis, namely, acute thrombosis after plaque rupture. Betaine is an organic compound used in clinical practice to lower homocysteine levels with a potential benefit for cardiovascular health.
Aim. To evaluate the effect of betaine on TF expression in human umbilical vein endothelial cells (HUVEC) and human mononuclear cells (MN). Methods: HUVEC were grown in 15% FCS 199/DMEM until confluency. MN were obtained from whole blood collected from healthy donors by Lymphoprep sedimentation. Cells were then incubated with betaine and the different reagents in various combinations at 37°C. At the end of incubation, cells were disrupted and procoagulant activity was assessed by a one-stage clotting assay and expressed in arbitrary units (U) by comparison with a standard preparation of human brain thromboplastin. TF mRNA was assessed by real-time RT-PCR.
Results. Betaine decreased the TF activity of HUVEC stimulated by bacterial lipopolysaccharide (LPS) in a dose-dependent way. The decrease was accompanied by a downregulation of TF mRNA. In the presence of other cell-activating agents such as IL1-β and TNF- α, a similar downregulation of TF activity and mRNA could be observed. Surprisingly, TF expression by MN exposed to LPS, as well as to TNF- α or IL-1 β, was unaffected by betaine. Inhibitory antibodies demonstrated that the activity was solely attributable to TF, which was expressed by the different cells at various degrees.
Conclusions. These data support the hypothesis that betaine, by its downregulation of TF, may play a role in processes underlying vascular cell disorders.

LIPOCHIP: DNA MICROARRAY APPROACH FOR THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA IN ITALIAN POPULATION

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Familial Hypercholesterolemia (FH) is a genetic disorder characterized by increased levels of total cholesterol, LDL cholesterol and early cardiovascular events. FH is mainly related to mutation in the gene coding for LDL-R which results in a protein with reduced to null efficiency in promoting LDL clearance form plasma. In addition to mutation in this gene FH has been associated less frequently with mutation in the genes coding for apolipoprotein B (ApoB) or for proprotein convertase subtilisin Kexin-9 (PCSK9). In the clinical setting, FH patient is identified according to the MEDPED score which encompass mainly the evaluation of the presence of elevated LDL cholesterol levels, presence of xanthoma, xanthelasma, early cardiovascular events, family history of CHD. Although this approach is very informative, the genetic diagnosis is the gold standard in the determination of FH. As the gene coding for LDL-R has several exons with reported mutations, this approach requires the amplification and sequencing of several amplicons thus needing long time for processing. More recently, the development of high throughput technologies such as DNA microarray approches, allowed to investigate the presence of several DNA sequences in a reduced time frame. The LIPOchip microarray encompass 3 hundred previously identified mutations mostly within the LDL-R but also for ApoB (exon 26) and PCSK9 and allows the identification of large insertions or deletion. We describe here the use of this approach for detecting the most frequent mutations associated with FH in the Italian population. The test was performed on a cohort of 101 DNA samples form patients with clinically diagnosis of FH from the various centers located throughout the country. The analysis allowed the identification of mutations associated with the LDL receptor in thirty-eight patients. In detail, there were frequent mutations in the population such as E207K, D200G the most applicants in northern Italy, the G528D and V502M with higher frequency in southern Italy. It ‘was also highlighted a mutation, the S156L, previously identified in Puerto Rico, but observed for first time in Italy.
Although we can not exclude that within the patients which did not show the presence of mutation either are present non FH subjects or FH subjects with previously unidentified mutations, the most probably explanation is that the version of the microarray used encompass only part of the most frequent mutations in Italy and therefore a more specific version for the Italian population is under preparation. In summary this approach is a fast and reliable alternative for the screening of the most common mutations associated with FH.

PLASMA EXCHANGE AND HETEROZYGOUS FH: A CASE REPORT

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Introduction. Plasma exchange is performed to selectively remove low density lipoprotein (LDL) from plasma and used for treatment of refractory familial hypercholesterolemia (FH). Case report Six patients, with high serum LDL-cholesterol (>500 mg/dl) and early cardiovascular disease (myocardial ischemia, internal carotid artery stenosis treated by endarterectomy or angioplasty with stenting), were admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples and treated with Plasma exchange. Clinical presentation, pedigree studies and molecular genetic diagnosis in one of them confirmed homozygous FH; in four patients, we found compound heterozygosis, while in last one a heterozygous status due to defective alleles in the LDL receptor gene. The heterozygous patient was 65 years old. He showed basal total Cholesterol of 700 mg/dl, HDL 57 mg/dl, Tg 196 mg/dl, LDL 603 mg/dl, tendinous xanthomatosis, recurrent myocardial infarctions (first event at 41 yr of age) treated by coronary artery bypass grafting despite intensive medical treatment; for this reason Plasma exchange was performed. This patient was underwent a careful monitoring of progression of atherosclerosis by ecocolor-doppler ultrasound, exercise electrocardiography and echocardiographic, and showed stable condition after two years.
Discussion. Plasma exchange showed an increase life-expectancy in homozygous FH. The main clinical indication has been homozygous FH (including compound heterozygous), but some studies showed that Plasma exchange could be effective on the progression of cardiovascular disease also in heterozygotes FH. The Japan Plasma exchange Coronary Atherosclerosis Prospective Study (L-CAPS) showed that a therapy with Plasma exchange combined with lipid lowering drugs can achieve a substantial decrease in LDL-cholesterol levels to induce a small but statistically significant regression or no progression of coronary artery lesions in heterozygous FH patients with severe coronary artery disease compared with progression in control FH patients with drug therapy. Plasma exchange improves the prognosis in FH patients. Plasma exchange therapy has 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 of intensive drug therapy .
Conclusion. Early Plasma exchange should be a useful tool also in heterozygous FH patients with high LDL-C levels, despite intensive drug therapy, or in patients with drug intolerance to prevent cardiovascular disease, its complications and to reduce atherosclerosis progression.

ROLE OF INFLAMMATORY MARKERS IN SUBCLINICAL ATHEROSCLEROSIS OF METABOLIC SYNDROME PATIENTS

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Objective. Metabolic Syndrome (MS) is a predictor of cardiovascular events and is associated with increased coronary and extracoronary atherosclerosis. In this study, we aimed at examining the ultrasound characteristics of atherosclerotic lesions in different arterial districts and their potential relation with several metabolic and inflammatory markers in individuals with the metabolic syndrome.
Materials and Methods. A total of 237 subjects with the metabolic syndrome (60±10 years, 156 men) and 233 age- and sex-matched healthy control subjects, all characterized by anthropometric variables (BMI, waist circumference), underwent ultrasound examination of the carotid and femoral arteries with the objective to measure intima-media thickness (IMT), degree of stenosis and echogenicity of plaques at different sites, flow-mediated vasodilation (FMV) and nitrate-mediated vasodilation (NMV) of brachial artery; total cholesterol, triglycerides, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, high sensitive C reactive protein (hs-CRP), glucose and insulin were also determined.
Results. In MS, the proportion of plaques was higher both at femoral and carotid site than in controls (85 vs 58%; $p<0.05$); dividing MS patients on the basis of FMV performance (normal: FMV& ≥10%; abnormal: FMV<10%) we found that patients with a higher prevalence of echolucent plaques had more frequently an abnormal FMV respect to those with prevalent echogenic plaques (74 vs 39%; $p<0.05$).
In a multivariate analysis, echolucency of carotid and femoral artery plaques was independently predicted by HDL cholesterol (odds ratio [OR] 0.7, 95% confidence interval [CI] 0.4-0.9), hs-CRP (OR 1.47, 95% CI 1.2–1.9), white blood cell count (OR 1.73, 95% CI 1.60-1.94) and FMV values (OR 1.61, 95% CI 1.4-1.8).
Conclusions. Subjects with MS show a high prevalence of echolucent, and potentially unstable, atherosclerotic plaques at carotid and femoral sites.
White blood cell count and FMV of brachial artery were independently related to the presence of echolucent plaques, suggesting their potential role in the cardiovascular risk stratification of subjects with the metabolic syndrome.

HYPOCHOLESTEROLEMIC EFFECT OF THE COMBINATION BETWEEN VEGETABLE PROTEINS AND FIBRES: IMPACT ON GENES REGULATING CHOLESTEROL HOMEOSTASIS

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Several clinical trials have demonstrated that total and LDL cholesterol levels can be efficiently decreased by integrating differ-

ent vegetable dietary ingredients. Aim of the present study was to compare, in an animal model, the hypocholesterolemic effect of individual and combined bioactive vegetable ingredients (pea proteins, oat fibres and apple pectin), and to investigate whether they could affect hepatic liver metabolism by regulating genes involved in cholesterol homeostasis. Six groups of 12 rats each were fed, for 28 days, Nath's hypercholesterolemic diets, differing for protein and fibre source being respectively casein and cellulose (control), pea proteins and cellulose (pea), casein and oat fibres (oat), casein and apple pectin (pectin), pea proteins and oat fibres (pea + oat), and pea proteins and apple pectin (pea + pectin). Each vegetable-containing diet determined a lower increase of total cholesterol levels vs controls at both 14 and 28 days of hypercholesterolemic diet. The two combinations (pea proteins + oat fibres and pea proteins + apple pectin) resulted more efficacious than fibres alone in modulating cholesterolemia (-72% and -74%, respectively at 28 days; $p<0.001$). Moreover, rats fed the diet containing oat fibres or apple pectin or the combinations had a lower cholesterol content in the liver ($p<0.005$) and higher hepatic mRNA concentrations of CYP7A1 than control rats ($p<0.05$). In the pectin group, the hepatic mRNA concentrations of HMG-CoA reductase and LDL receptor were increased compared to control group ($p<0.05$). Rats fed pea proteins had higher hepatic mRNA levels of ABCG8 than those fed casein ($p<0.05$). In conclusion, pea proteins, oat fibres and apple pectin markedly modulate cholesterol increase in rat plasma. The two combinations are more efficacious than fibres alone. Moreover, our results suggest that these bioactive ingredients affect cellular cholesterol homeostasis by up-regulating genes involved in hepatic cholesterol turnover. Funding: CRAFT Project Bioprofibre (COOP-CT-2006-032075)

CORRELATION BETWEEN GLOMERULAR FILTRATION RATE (GFR) AND ARTERIAL BRACHIAL INDEX (ABI) IN DYSLIPIDEMIC SUBJECTS: OUR EXPERIENCE

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Background. The early detection of GFR<60 ml/min and an ABI<0.9 are considered two stages of basic clinical practice to predict cardiovascular risk. We decided to detect the correlation between GFR fir in subjects related to a specialized clinic devoted to the diagnosis and treatment of dyslipidemia.
Methods. We evaluated in our Lipid Center, a cohort of 1748 dyslipidemic subjects (mean age 53 aa., 912 F, 836 M). For all, at first visit was observed by the GFR, estimated with the formula Cockoft and Gault, and both the ABI. For all, beyond the clarification of the specific diagnostic primitive form of dyslipidemia, was found to contain any other cardiovascular risk factors: diabetes, hypertension and the presence of overt ischemic heart disease (CHD).
Results. In all of our series, the GFR was <60 ml/min in 17% of cases fir was <0.9 in 13% of cases. Dyslipidemia in patients presenting with a GFR <60 ml/min ABI<0.9 was present in 49% of cases. In subjects with GFR<60 ml/min and the percentage of diabetes patients with ABI<0.9 rising to 49%. In patients presenting CKD, diabetes and hypertension ABI<0.9 was present in 89% of cases. In 50% of subjects, in addition to diabetes and dyslipidemia, were also present a GFR<60 ml/min and an ABI<0.9, ischemic heart disease was already present.
Conclusion. We believe that our data on increasing prevalence of abnormal ABI in the course of CKD should be to reinforce the belief that early detection of GFR and ABI should get into habits of common clinical practice, being able to give ipso facto an important information already at the first visit, on cardiovascular risk of the person concerned, especially when it arises in a state of complexity dysmetabolic.

CAROTID INTIMA-MEDIA THICKNESS AND BONE TURNOVER MARKERS

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Patients with osteoporosis frequently suffer from vascular calcification, which predicts both cardiovascular morbidity and mortality, and osteoporotic fractures. Several studies show that aortic calcifications are positively associated with osteoporotic fractures both in women and men, and the progression of vascular calcifications is positively related to the rate of decline of bone mineral density (BMD). Similarly, common carotid artery intima-media thickness (CCA-IMT), which is known to be associated with an increased risk of myocardial infarction and ischemic stroke, is negatively linked to the lumbar spine BMD in postmenopausal women. Nevertheless, the relationship between osteoporosis and atherosclerosis remains incompletely understood. Several common mechanisms and risk factors have been suggested to contribute to the development of osteoporosis and atherosclerosis.
The present cross-sectional study was performed to determine whether the degree of bone turnover is correlated to carotid intima-media thickness (CCA-IMT), as a marker of subclinical atherosclerosis. We studied 50 outpatients (mean age 71.7±12.3), underwent to eco-Doppler evaluation of extracranial carotid tract, without history of calcium and/or vitamin D supplementation, or antireabsorptive therapy. CCA-IMT was measured by high-resolution B-mode ultraso-nography.
Bone turnover was evaluated by analysing serum levels of C-terminal telopeptide of type I collagen (sCTX), and bone-specific alkaline phosphatase.
We also evaluated the vitamin D status by determination of the serum concentration of 25-hydroxyvitamin D [25(OH)D]. We found a prevalence of 91.8% of hypovitaminosis D [serum 25(OH) D levels < 30 ng/mL] with a mean value of 10.7±5.8 ng/mL. Eighty eight percent of the whole population had a CCA-IMT value > 0.9 mm, with a mean value of CCA-IMT of 1.55±0.7 mm. An increased bone resorption, with mean sCTX levels of 1.18±0.57 ng/mL were also observed. A significant positive correlation was found between CCA-IMT and age ($r=0.480$, $P=0.001$), erythrocyte sedimentation rate (ESR: $r=0.438$, $P=0.001$), high-sensitivity C-Reactive Protein (HsCRP: $r=0.482$, $P=0.011$), serum creatinine ($r=0.305$, $P=0.031$), and sCTX ($r=0.389$, $P=0.006$).
In a multivariate linear regression, CCA-IMT was independently predicted by age (beta=0.34, $P=0.001$), ESR (beta=0.37, $P=0.005$), and sCTX (beta=0.32, $P=0.006$).
The preliminary results of our study seem to indicate that sCTX levels may predict carotid wall thickening in an elderly population, and may represent one of the possible common pathogenetic mechanisms responsible for the simultaneous and frequent presence of osteoporosis and arteriosclerosis in these subjects.

ATORVASTATIN INHIBITS OXIDATIVE STRESS VIA ADIPONECTIN-MEDIATED NADPH OXIDASE DOWN REGULATION IN HYPERCHOLESTEROLEMIC PATIENTS

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Background. Interventional treatment with atorvastatin lower the circulating levels of the catalytic core of NADPH oxidase, namely sgp91phox but the underlying mechanism is still undefined. Previous studies showed that patients with hypercholesterolemia have impaired adiponectin production and that atorvastatin is able to up-regulate it. In vitro studies suggested that adiponectin possesses antioxidant properties and hypothesized an involvement of NADPH oxidase.
Aim. We test the hypothesis that the inhibitory effect on oxidative stress, induced by Atorvastatin, could be mediated in vivo by adiponectin.
Methods and Results. We compared 36 patients with polygenic hypercholesterolemia and 18 healthy subjects. Patients were randomized to either a diet or atorvastatin 10 mg/day for 30 days. Lower serum adiponectin levels and higher lipid profile, gp-91phox serum levels, urinary isoprostanes, platelet oxygen free radicals, characterized patients. After 30 days of atorvastatin treatment patients showed higher levels of adiponectin which is inversely correlated to reduced levels of sgp91phox , urinary isoprostanes and platelet oxygen free radicals ($p<0.001$). Values of adiponectin, sgp91phox , urinary isoprostanes and platelet oxygen free radicals were unchanged at day 30 in the diet allocated group.
We also studied in vitro p47phox translocation from cytosol to cell membrane to evaluate the direct effect of adiponectin on NADPH oxidase activity. This test is a direct probe of NADPH oxidase activation as the cytoplasmatic subunit p47phox binds to the membrane subunit gp91phox only upon the enzymatic system activation. Gp91phox concentration on platelet membrane and sgp91phox in the supernatant of activated platelets were also evaluated. Adiponectin, dosages between 5 and 10 ng/ml, inhibited p47phox translocation to gp91phox and soluble gp-91phox cleavage indicating its ability in inhibiting NADPH oxidase activation.
Conclusion. This study provides the first evidence that higher adiponectin serum levels are associated with gp91phox down-regulation in hypercholesterolemic patients and that atorvastatin exerts an antioxidant effect via adiponectin-mediated NADPH oxidase inhibition.

ENDOTHELIAL DYSFUNCTION IN CHRONIC RENAL FAILURE

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Cardiovascular disease is a very early phenomenon in the course of chronic renal failure, and increases continuously with decrease of renal function. Endothelial dysfunction seems to be a starting point in vascular changes leading to atherosclerosis and artery calcification. Endothelium, considered the largest organ in the body, has many functions. It senses mechanical and hormonal stimuli and in response the endothelial cells secrete a range of compounds which modulate vascular tone, coagulation, cell proliferation, and inflammation. The central role of endothelium in the development of vascular disease has led to identification of new relevant biomarkers and methods to estimate endothelial function and injury. Arterial stiffness, which is not an early phenomenon in endothelial dysfunction but a common complication of chronic renal failure may be evaluated through Pulse Wave Velocity obtained by pulse-wave analysis. The aim of the present study was to analyze correlations between arterial stiffness and erectile dysfunction evaluated with International Index of Erectile Function 15(IIEF-15), in the predialysis group and compared it with healthy controls. Supine brachial artery systolic(SBP)and diastolic blood pressure(DBP)were measured with an oscillometric device. PWV was measured by recording pulse waves at right common carotid artery (CCA) and artery sequentially using tonometry and simultaneous electrocardiogram.
The subjects were divided in 3 groups, according to PWV values. Our data from 93 subjects(54 males)showed a positive correlation between erectile dysfunction(low score of IIEF)and higher values of PWV.In conclusion, arterial calcification is present in patients with CKD, and inflammation is a common phenomenon in uremic patients. Arterial stiffness and erectile dysfunction represent two face of the same pathology: endothelial dysfunction. The application of this evaluation to patients with CRF, may allow to predict cardiovascular events and to make prevention in this subgroup of patients.

AWARENESS OF CARDIOMETABOLIC RISK FACTORS AMONG EMPLOYERS IN TREVIGLIO HOSPITAL

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Aim of the Study. In order to analyse the health status of Treviglio Hospital staff, we carried out an evaluation of some cardiovascular risk factors collecting data about their weight and the role played by nutrition in their health conditions. We also tried to find some differences among the four components of the Hospital Company (Treviglio-Caravaggio, Romano di Lombardia, Calcinate, S. Giovanni Bianco), to check the degree of sedentariness and physical activity practiced by people working in our Hospital, and to verify that the BMI declared data corresponded to those collected by the internal physician. The final aim was to

propose practical behaviours achievable at the workplace, which should increase consciousness and awareness of obesity and physical inactivity as risk factors to develop both diabetes and metabolic diseases. In this way, employers themselves could become the best testimonials and promoters of a healthy lifestyle.
Materials and Methods. We chose a structured questionnaire allowing us to quantitatively evaluate the research hypothesis and to develop a descriptive analysis. 651 questionnaires were uniformly distributed to the Hospital staff (2000 units) and collected from all the sanitary and administrative areas. The 80% of the answers came from people employed in the sanitary area, who are on average 45 years old (range 30-60). This sample was regarded as statistically descriptive of the universe investigated population.
Results. The Hospital staff, due to its own activity, is regarded to be more informed than the general public about obesity, its metabolic consequences and the importance of a regular physical activity. Nevertheless, this awareness doesn't always correspond to the acquisition of correct behaviours.
Conclusions. It would be therefore necessary to create new motivational strategies to promote a healthy lifestyle as first prevention of diabetes and metabolic disorders. A first proposal was the institution of a training course via web available from the hospital portal for all the employers.

INCREASING OF HDL CHOLESTEROL LEVELS IN COMBINED DYSLIPIDEMIC PATIENTS IS ASSOCIATED TO AN IMPROVEMENT OF FLUX-MEDIATED DILATATION

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Introduction. Familial Combined Dyslipidemia (FCH) represents one of the major risk factors for cardiovascular disease. The 10 % of recovers in coronary unit is actually represented by FCH patients. Low HDL cholesterol values and hypertriglyceridemia, characterising these subjects, are often associated with a peripheral vascular dysfunction.
Objective. Aim of the study was to assess if the amelioration of dislypidemia, due to statins theraphy, observed for FCH patients was related to endothelial function measured as flux-mediated dilatation (FMD) at the brachial artery.
Methods. In 35 FCH patients (M/F.20/15), before and after statins therapy, we evaluated: HDL cholesterol, triglyceridemia, HDL/ApoA1 cholesterol, triglyceridemia/cholesterol HDL and FMD.
Results. After six months of therapy we observed an increased level of HDL cholesterol (40,34 ± 11,13 vs. 51,13 ± 11,95, P<0.03), a reduction of triglyceridemia (240,56 ± 111,76 vs. 158,36 ± 118.09, P<0.01) an increase of HDL cholesterol/ApoA1 ratio (0,27 ± 0,05 vs. 0,32 ± 0,19, P<0.03), a reduction of trig/HDL ratio (5,57 ± 3,3 vs. 3,51 ± 3,5, P<0.03) and an increase of FMD (21,05% ± 9,3 vs. 26,3% ± 6,49, P<0.05).
Conclusions. Amelioration of dislypidemia in FCH patients results associated with an improvement of vascular function, measured as FMD, suggesting a functional association between FMD, HDL cholesterol and triglyceridemia.

RELATIONSHIPS BETWEEN CAROTID ATHEROSCLEROSIS AND BONE MINERAL DENSITY IN NORMAL SUBJECTS AND IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Although osteoporosis and atherosclerosis have always been considered as separate entities, there is accumulated evidence that they have several pathological mechanisms in common. However at present very scarce data exists about any relationships between osteoporosis and carotid atherosclerosis. This study aimed to evaluate if carotid atherosclerosis was correlated with bone mass density (BMD) in normal subjects and in subjects with type 2 diabetes mellitus (DM2). In 273 subjects (mean age 56±7.5 years), 178 normal subjects and 95 patients with DM2, we measured Bone Mineral Density (BMD) at lumbar spine (BMD-LS), at femur (neck: BMD-FN; total: BMD-FT) and at total-body (BMD-WB). An ultrasound examination of carotid vessels was performed to assess intima-media-thickness (IMT), presence of plaque and the degree of calcified. As expected, BMD resulted significantly higher in subjects with DM2 than in normals.
A significant inverse correlation was found between BMD-FN and BMD-FT with IMT (r=-0.10, p=0.09 and r=-0.15, p=<0.05 respectively), with carotid stenosis (r=0.19, p<0.05 and r=-0.15, p< 0.05) and with plaque score (r=-0.18, p<0.05 and r=-0.14, p<0.05). BMD-LS and BMD-WB, showed an inverse relationship only with stenosis percentage.
Relationships between BMD and carotid atherosclerosis resulted lower in DM2 than in normal subjects. In DM2 patients stenosis percentage and calcification degree were inversely correlated only with BMD-FN (respectively r=-0.17, p<0.05 and r=-0.24, p 0.01). In both normals and DM2 patients the presence of osteoporosis (T-Score <-2.5) was significantly associated to a more severe carotid atherosclerosis. Our study confirms that DM2 can cause a marked increase in carotid atherosclerosis. Moreover, in both normal subjects and patients with DM2 a lower BMD was associated to a more severe carotid atherosclerosis and to a higher calcium content in plaques.

NONALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH AN INCREASED PREVALENCE OF CARDIOVASCULAR DISEASE IN TYPE 1 DIABETIC SUBJECTS

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Background. Nonalcoholic fatty liver disease (NAFLD) is associated with an increased prevalence of asymptomatic/symptomatic cardiovascular disease (CVD) in both patients without diabetes and in those with type 2 diabetes. Information on this issue is lacking for type 1 diabetes. The aim of this study was to assess whether NAFLD is associated with an increased preva-

lence of asymptomatic/symptomatic CVD in type 1 diabetic individuals.
Methods. We studied 343 (156 men and 187 women; mean age 44 years) adult patients with diagnosed type 1 diabetes with and without NAFLD (as detected by liver ultrasound), who consecutively attended our diabetes outpatient clinic over the last two years. Asymptomatic/symptomatic CVD was detected by patient history, chart review, electrocardiogram and echo-Doppler scanning of the carotid and lower limb arteries in all participants.
Results. Patients with ultrasound-diagnosed NAFLD (n=182) had a remarkably greater prevalence of coronary (15.4 vs. 1.2%; p<0.0001), cerebrovascular (76.4 vs. 33%; p<0.0001) and peripheral (48.9 vs. 10%; p<0.0001) vascular disease than did their counterparts without NAFLD (n=161). A multivariate logistic regression analysis revealed that NAFLD was associated with an 11-fold higher odds of CVD (considered as a composite endpoint) independently of age, sex, body mass index, diabetes duration, hemoglobin A1c, family history of CVD, physical activity, smoking status, systolic blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides, estimated glomerular filtration rate, albuminuria and the use of anti-hypertensive, anti-platelet or lipid-lowering medications (adjusted odds ratio 11.3, 95% confidence intervals 5.4-24.1, p<0.0001).
Conclusions. Our findings suggest that ultrasound-diagnosed NAFLD is associated, independently of several potential confounders, with an increased prevalence of CVD in type 1 diabetic individuals. Future prospective studies are needed to determine whether NAFLD may contribute to the development of CVD in this patient population.

IN VITRO FUNCTIONAL CHARACTERIZATION OF NOVEL AMINO ACID SUBSTITUTIONS IN PCSK9 FOUND IN HYPOCHOLESTEROLEMIC SUBJECTS

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Introduction. Proprotein convertase subtilisin kexin type 9 (PCSK9) is a glycoprotein expressed mostly in liver, kidney and intestine. It is synthesized as a proprotein which undergoes autocatalytic cleavage in the endoplasmic reticulum before being secreted as mature PCSK9 (enzimatically active). The normal function of PCSK9 is to accelerate the turnover of LDL receptor (LDLr) in the liver regulating the number of LDLr and consequently the plasma level of LDL-cholesterol. Mutations in PCSK9 gene may increase (gain of function mutations) or decrease (loss of function mutations) the function of PCSK9 protein resulting in hypercholesterolemia and hypocholesterolemia respectively.
Objectives. Aim of this study was the in vitro characterization of the biological effect of rare non-conservative amino acid PCSK9 substitutions identified in hypocholesterolemic subjects.
Methods and Results. To define the effect of non-conservative amino acid substitutions T77I, V114A, P345L e A522T in PCSK9 protein (which in principle could result in a loss of function of PCSK9) expression plasmids containing human PCSK9 cDNAs harbouring the naturally occurring missense mutations were transiently transfected in HepG2 and HuH7 cells. PCSK9 protein in cells and media and the cellular content of LDLr protein were measured. As compared to wild type PCSK9 the V114A, P345L and A522T mutants showed a 50% reduction of PCSK9 in the media and an increased amount of intracellular PCSK9, notably of the proprotein form. These changes were associated with a 1.5-2 fold increase of the amount of cell LDLr protein. The T77I mutant showed an effect similar to that of wild type PCSK9.
Conclusions. These data suggest that the new missense mutations of PCSK9 (with the exception of T77I) found in hypocholesterolemic individuals are to be considered novel loss of function mutations.

DUAL EFFECT OF HYPOCHLORITE IN THE MODIFICATION OF HIGH DENSITY LIPOPROTEINS

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HDL-cholesterol levels are inversely correlated to the risk of atherosclerotic disease. In the recent years the concept that not only quantity, but also quality of HDL particles is essential for their protective role became apparent. Many studies have showed that, under particular pathological conditions, HDL can be modified, shifting their property from anti-atherogenic to pro-atherogenic. However, not all kind of modifications are deleterious for HDL properties. For example, tyrosylation of HDL improves its ability to remove cholesterol from cultured cells and inhibits mice atherosclerotic lesion formation; oxidation of HDL3 with 15-lipoxygenase or with copper ions for short time induce the formation of pre-ß-migrating particles that confers a better function as cholesterol acceptor. Myeloperoxidase modifies HDL and apoA-I and reduces their ability to promote ABCA1-mediated cholesterol efflux.
In the present work we show that modification with low concentration HOCl (5 µM), a myeloperoxidase product, induces the formation of pre-ß-migrating particles, thus improving the function of HDL in the ABCA1-mediated cholesterol transport (6,32±0,76% for HOCl-modified HDL3, 3,48±1,16% for HDL3, p<0.001), without affecting the ability to decrease TNFα-induced adhesion molecule expression.
At higher HOCl concentration (250 µM), pre-ß-migrating particles were not formed, ABCA1-mediated cholesterol efflux was unchanged while SR-BI-mediated cholesterol efflux was reduced (22,56±0,39% for HDL3, 18,43±0,36% for HOCl-modified HDL3, p<0.0005); furthermore the anti-inflammatory property was negatively affected.
These findings suggest that during early inflammation, low HOCl concentration are generated that can induce changes in HDL3 able to increase their ability to remove cholesterol; later during acute inflammation, higher HOCl concentration will induce changes in HDL that decrease their ability to remove cholesterol from macrophages and to protect endothelial cells from pro-inflammatory stimuli.

CIRCULATING OSTEOBLASTS CONTRIBUTE TO AORTIC CALCIFICATIONS AND STIFFNESS IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS

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Introduction. Increased arterial stiffness and ectopic artery calcification, two early markers of atherosclerosis and cardiovascular risk, have been documented in women with postmenopausal osteoporosis. Also, an imbalance in the number of circulating osteoprogenitor cells (OPCs) expressing bone-related proteins has been identified in postmenopausal osteoporotic women. We investigated whether an association exists between aortic calcifications and arterial stiffness and the influence of circulating OPCs on vascular calcification process in postmenopausal osteoporosis.
Methods and Results. The number of circulating OPCs was quantified by FACS analysis in 32 newly diagnosed osteoporotic postmenopausal women, not carrying traditional cardiovascular risk factors except for age. OPCs were defined as CD15 negative/alkaline-phosphatase(AP)+ cells. Participants underwent cardiovascular risk factor assessment, measurement of bone mineral density, aortic pulse wave velocity (PWV) as a measure of arterial stiffness, and aortic calcium score by low dose 64-slice computed tomography. Among osteoporotic postmenopausal women, aortic PWV was significantly associated with aortic calcium score (rho=0.73, p<0.001). Other significant correlates of aortic calcium score included age (rho=0.74, p<0.001) and calcium (rho=0.52, p=0.02). Also, the number of CD15 negative/AP+ OPCs was positively associated with aortic calcification score (rho=0.67, p=0.01). In multivariate regression analysis the model including age, calcium, triglyceride levels and the log-transformed number of CD15 negative/AP+ cells explained 72% variability of aortic calcium score, being the number of circulating OPC's a significant independent predictor of aortic calcium score. Also, OPCs number was a significant predictor of aortic stiffness, independent of other PWV covariates.
Conclusions. In women with postmenopausal osteoporosis, increased arterial stiffness may be significantly explained by an increased ectopic arterial calcification, which in turn is strongly influenced by the availability of circulating osteoprogenitor cells.

TWO NOVEL ITALIAN MUTATIONS OF APOA5 GENE CAUSING FAMILIAL HYPERTRYGLICERIDEMIA

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Family 1. The proband was a 40 year-old male from Locri (RC), BMI 24.4 kg/m^2, with mild hypertriglyceridemia (HTG) from 30 years of age (TC 5.09, HDL-C 0.57, TG 7.33 mmol/L). A previously reported TG level was 13.5 mmol/L. His HTG mother suffered from pancreatitis at 55 and died at 64 for acute renal failure.
Family 2. The proband was a 31 year-old male, BMI 32.0 kg/m^2, with severe HTG (TC 5.92, HDL-C 0.57, TG 11.73 mmol/L, Apo AI 110 and Apo B 100 mg/dL.
The proband's father, from Cile, was 58, BMI 29.3 kg/m^2 and had moderate HTG (TC 6.36, HDL-C 0.98, TG 6.22 mmol/L). Genetic analysis was performed, in the probands, sequencing LPL, APOC2, APOA5, GPI-HBPI and LMF-1 genes and determining APOE genotype, and some APOA5 and APOC3 allelic variants.
Results. Proband 1 was heterozygous for a deletion in exon 4 of APOA5 gene (c.293-295 del; p.E98 or E99 del). He had e3e3 genotype, was homozygous for the rare APOC3 allele –482 T and heterozygous for APOA5 –1131 T/C. E99 in strictly conserved in all species while E98 is less conserved. The proband 2 was heterozygous for the common APOA5 variant p.S19W (inherited from the mother) and for a APOA5 missense mutation in exon 4 (c.758 T>C, p.L253P; inherited from the father). In silico analysis of the mutation with Polyphen, SIFT, Pmut and SNPs3D suggested its pathogenic effect in all cases. He was e3e3 and heterozygous for the APOC3 –482 T/C SNP.

LEUCINE 10 AND 11 ALLELIC VARIANTS IN SIGNAL PEPTIDE OF PCSK9 INCREASE THE LDL CHOLESTEROL LOWERING EFFECT OF STATINS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Objective. Carriers of an additional leucine residue in a stretch of 9 leucines in the signal peptide of PCSK9 (L10) have lower total (TC) and low density lipoprotein-cholesterol (LDL-C) than homozygotes for the wild type allele (L9/L9). A similar effect was detected in FH patients with the p.C681X mutation of LDL-receptor (LDLR). We investigated the effect of L10 and L11 variants on basal lipid profile and response to statins in molecularly characterized FH patients.
Methods. Plasma lipids were determined 322 FH patients screened for the L9/L10/L11 polymorphism and in a subgroup of 54 patients carrying the same LDLR mutation (p.Q474H>Fs>X536). Plasma lipids were also determined in 40 FH patients carrying the L10 or L11 variants and in a parallel group of 40 FH patients, L9/L9 homozygotes, matched for gender, age, type of LDLR gene mutation, and for type, dose and duration of statin treatment.
Results. In FH patients no difference in the basal plasma TC and LDL-C levels were observed between carriers of L10-L11 variants (L9/L10 + L10/L10 + L9/L11) and L9/L9 homozygotes. The same was true in FH patients carrying the p.Q474H>Fs>X536 LDLR mutation. In the subgroups of statin treated patients the reduction of TC and LDL-C was greater in carriers of L10 or L11 (-33.4% and -41.8%, respectively) than in L9/L9 homozygotes (-24.9% and -31.2%, respectively) (P<0.001).
Conclusion. The variants of the leucine repeats in PCSK9 signal peptide are to be considered as factors capable of modulating the lipid-lowering effects of statins in FH.

A RARE CASE OF SEVERE HYPERCHILOMICRONEMIA AND NEUROLOGICAL ABNORMALITIES ASSOCIATED TO A NOVEL MUTATION OF LPL GENE

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The proband was a 2 month-old female with severe hypertriglyceridemia (TG 32.5 mmol/L) and low total cholesterol (1.7 mmol/l). Clinical examination was abnormal with hypertonia of all limbs. BMI was normal in relation to age, xanthomatas and lipaemia retinalis were absent. MRI of brain was abnormal with agenesis of corpus callosum and cerebellar hypoplasia. Parents were consanguineous Pakistani, their triglycerides were normal, without clinical history of pancreatitis. One proband's brother presented a similar clinical features (neurological abnormalities from the first year of life and hyperlipidemia) but his MRI was different from the proband; his twin sister was also abnormal and died in Pakistan at age of 7 y. Two other sibs were in good health. Even if a similar case had been described in a child with APOC2 deficiency (Wilson C.J. et al. Ann Neurol 2003: 53:807-10), at first we analyzed LPL gene and identified the genetic defect. The proband was homozygous for a complex rearrangement in exon 3: c.289-294 delGCCGCC and c.288 insTTTGCCAAAA. The mutation causes a frameshift at position 97 of the LPL pro-protein and introduces a termination codon at position 148 (p.Ala97Phe>Fs>Term148). The study of the other family members is in progress.

DOES STATIN AFFECT CAROTID PLAQUE COMPOSITION INDEPENDENTLY FROM LDL REDUCTION? COMPARISON OF EXPERIMENTAL AND CLINICAL TRIALS

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The impact on cardiovascular events achieved by statin therapy seems to be mostly attributable to the cholesterol-lowering effect with a highly debated contribution of the lipid-independent pleiotropic effects. Experimental studies support the concept that statins have anti-inflammatory properties. However, in animal studies atherosclerotic disease is studied in the initial stages while in humans plaques are removed when advanced lesion are present, moreover plaque cellular composition vary substantially between species.
Main nonlipid-related beneficial properties of statins include protective effect on endothelial function, antithrombotic actions, and anti-inflammatory effects. In vitro and experimental studies have shown that there are common mechanism between the LDL-C-lowering and the pleiotropic effects of statin therapy. This is further supported by the meta-analysis of Kinley that clearly highlights that most of the anti-inflammatory effects of LDL-lowering therapies are related per se to the magnitude of change in LDL-C.
In humans only few studies examined the impact of statin therapy on carotid plaque composition. Some retrospective large studies using plaque specimens have been reported. In those studies heterogeneity of designs, selection of patients and treatments could explain the conflicting results. A previous prospective study by Crisby and coll., was carried out in 13 patients undergoing carotid endarterectomy after a three month pravastatin treatment, showed a lower macrophage and lymphocyte content of the plaque along with a reduced accumulation of lipids compared to arteries from control subjects that were not treated with lipid lowering therapy. Recently we studied how different lipid-lowering strategies (non-statin therapy, low-dose statin and high-dose statin) affect cellular composition of carotid plaque. We recruited 60 hypercholesterolemic patients eligible for carotid endarterectomy. Three months prior surgery patients were randomized into 3 groups receiving atorvastatin 10 mg/day, or atorvastatin 80 mg/day, or cholestyramine 8 g/day plus sitosterol 2.5 g/day. We have found that short-term treatment with high-dose statin is superior to a non-statin lipid lowering regimen in reducing the macrophage cell content inside atherosclerotic lesions, and this effect is significantly modulated by the degree of LDL-C lowering. Adjustment of the outcome results for the lowering of LDL levels blunted the differences on plaque macrophage concentration among groups, although a clear trend was still observed. To further define the lipid-dependent versus a nonlipid-dependent (pleiotropic) effect of statins on plaque cell composition, a larger cohort of patients might be required, highlighting a main limitation of such studies in humans.
Infact, a recruitment of a larger high-risk and lipid-lowering naïve population is now limited by the current standard of treatment. In conclusion, cellular plaque composition even after a short-term lipid lowering therapy is significantly modulated by the degree of LDL-C lowering. A contribution of LDL independent, anti-inflammatory mechanisms of statins on plaque stability is only suggested by studies. Actually, data strongly support the current guidelines based on progressively lower LDL-C targets depending upon the cardiovascular risk of individual patients.

SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH PSORIATIC ARTHRITIS

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Objective. Increased cardiovascular morbidity and mortality have been observed in several immune mediated rheumatic diseases, including psoriatic arthritis (PsA). We evaluated vascular remodelling in PsA patients by studying non invasively structural and functional properties of arteries. Since hypertension may represent a main confounding, PsA patients were stratified according to the presence/absence of hypertension as defined by standard criteria.
Design and Method. We studied 41 consecutive patients with PsA (of whom, 23 hypertensives) attending hospital outpatient clinics who fulfilled the Moll and Wright criteria will be included. Traditional and non traditional risk factors for atherosclerosis and clinical variables were evaluated. 40 normotensive

healthy subjects (NC) and 24 hypertensive subjects (HT-C), comparable by age and sex, served as controls. Subclinical atherosclerosis will be assessed by non-invasive methods that analyze the structural and functional properties of the arterial wall. We evaluated by B-mode ultrasound the carotid intima media thickness (IMT). Measurements were expressed as mean-IMT (cumulative mean of mean IMT measured in each carotid segment, common, bulb, and internal carotid artery, bilaterally) and as M-MAX (cumulative mean of maximum IMT). Endothelial function was evaluated by post-occlusion flow mediated dilation (FMD) of the brachial artery using high-sensitivity brachial ultrasonography. NO-independent vasodilation was evaluated by the response to sublingual glyceril trinitrate (GTN).
Results. PsA had a higher mean-IMT compared to NC. Hypertensive PsA displayed higher M-MAX versus both HT-C (p=0.007) and normotensive PsA (p=0.026). FMD was significantly lower in PsA than in NC (8.9%), whereas there was no difference between hypertensive PsA (6.1%), normotensive PsA (5.7%), and HT-C (6.3%). GTN response was similar in all groups. The TNF-alpha level was much higher in PsA patients than in the other groups. High sensitivity-CRP was higher in PsA patients but in our series was not related to neither IMT or FMD measurements. Values of serum fibrinogen, IL-6 and VEGF resulted comparable in the studied groups. In the entire cohort, the IMT parameters were significantly related to TNF-alpha as well as classical risk factors, including blood pressure and lipid profile, whereas FMD was inversely related to TNF-alpha levels and blood pressure but not lipid parameters.
Conclusions. Subclinical atherosclerosis is enhanced in PsA compared to NC. In PsA, the hypertensive status proved to exert an additional effect on M-MAX, a parameter of advanced pro-atherogenic remodelling. FMD was reduced in PsA irrespective of hypertensives status. Thus, PsA per se implies a pro-atherogenic remodelling which is enhanced by the hypertensive status. In addition, TNF-alpha seems to play a role in the hampering the functional properties of vascular wall probably through endothelial dysfunction.

COMBINED EFFECTS OF OFFICE AND 24-HOUR BLOOD PRESSURE ON AORTIC STIFFNESS IN HUMAN HYPERTENSION

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Aortic stiffness, a prognostically adverse marker of arteriosclerosis, is critically dependent on blood pressure (BP). However, BP values measured in the office may not always reflect BP behaviour away from the medical environment, and it is uncertain whether office or out-of-office BP values are stronger determinants of arterial stiffness. In 539 untreated patients with uncomplicated essential hypertension and 71 normotensive subjects, we measured 24-hour BP and carotid-to-femoral pulse wave velocity (PWV), a direct measure of aortic stiffness. Aortic PWV was lower in normotensives than in white-coat hypertension (8.4+-2 vs 9.3+-2 m×s-1, p=0.019) and in sustained hypertension (9.8+-2 m×s-1, p<0.001).
To examine the independent effect of office BP on aortic PWV beyond the influence of 24h BP, subjects were classified according to the difference between observed and predicted office systolic BP (the latter determined by regressing 24-hour BP on office BP).
Despite having comparable 24-hour BP values (131/82 vs 131/84 mmHg), the subjects with higher-than-predicted office BP had higher aortic PWV than the subjects with lower-than-predicted office BP (10.1+-2 vs 9.2+-2 m×s-1, p<0.001). Similarly, after regressing office BP on 24-hour BP, we obtained 2 groups with identical office BP (152/95 vs 152/96 mmHg) but different 24-hour BP.
The group with higher-than-predicted 24-hour BP had significantly higher aortic PWV (9.9+-2 vs 9.5+-2 m×s-1, p<0.05). In a multiple regression model, both 24-hour and office mean BP were independent predictors of aortic PWV. In conclusion, both office and out-of-office blood pressures are independent predictors of aortic stiffness in hypertension.

BRACHIAL SYSTOLIC AND DIASTOLIC BLOOD PRESSURE AT DIFFERENT ARM HEIGHTS: A NOVEL INDEX OF ARTERIAL FUNCTION

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Systolic and diastolic blood pressure (BP) changes over different mean pressure levels has been used to generate the (ambulatory) arterial stiffness index, and may reflect functional arterial properties. We hypothesized that pressure changes obtained by changing arm position may represent a tool to investigate arterial function at bedside. In 56 healthy subjects (age 48±18 years, BP 125/71±18/10 mmHg), we measured carotid-radial pulse wave velocity (PWV) and sitting brachial BP (12 readings with the arm in 4 different positions, 3 readings per position). SBP-on-DBP slope, estimated by the ratio of their standard deviations, was defined as BPVR (BP variability ratio). Recent model expresses BPVR as the systolic-to-diastolic stiffness ratio. Diastolic stiffness was expressed by PWV2 (Bramwell-Hill formula). As expected from Stevin's law, mean pressure changed linearly with the cuff-heart vertical distance (-14 mmHg, -8 mmHg, and +9 mmHg, respectively, at +20, +10 and -15 cm; p for linear trend <0.001).
Diastolic PWV2 had a linear relationship with DBP (r=0.40, p=0.005). Also, calculated systolic stiffness (BPVR × diastolic PWV2) had a direct relationship with SBP (r=0.60, p<0.05). BPVR had no relation with PWV2 (r=-0.18, p=n.s.), and a strong one with age (r=0.45, p<0.01) and Framingham coronary risk (r=0.60, p<0.001). In conclusion, SBP/DBP changes at different arm heights may provide a novel measure of arterial function. The resulting SBP-on-DBP slope had no correlation with diastolic arterial stiffness, and increased with increasing SBP, age and estimated coronary risk. Results support the theoretical expression of SBP-on-DBP slope as the ratio between systolic and diastolic stiffnesses.

EPICARDIAL FAT THICKNESS IS AN INDEPENDENT PREDICTOR OF LEFT VENTRICULAR REPOLARIZATION CHANGES

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Background. Obesity is strongly linked to the development of diabetes, insulin resistance, and cardiovascular diseases, and body fat distribution may play a key role in the above associations. Fat excess may accumulate in non-adipose ectopic sites, as abdominal viscera, liver, heart, and muscles. Ectopic and particularly cardiac fat tissue has been related to an increased cardiovascular risk, independently of total body fat mass. Unlike pericardial fat, epicardial fat is contiguous with myocardium and shares with it the same coronary microcirculation. Epicardial adipose tissue actively produces and secretes several bioactive adipokines which have been involved in the pathogenesis of atherosclerosis. Because of its proximity with myocardium and the absence of anatomic boundaries, such mediators may interact locally with coronary vessel wall and exert vasocrine and paracrine effects which might have a role in the development of coronary artery disease (CAD).
Epicardial fat thickness, in fact, has been positively associated with CAD in different settings and populations independently of visceral fat, and has a significant relation with reduced coronary flow reserve in women. 2D-echocardiography can accurately measure epicardial fat thickness and has the potential to distinguish it from pericardial fat. Hypertensive individuals are at increased risk for CAD. Resting electrocardiography (ECG) may detect CAD in a very small proportion of subjects. Minor ST-T abnormalities are encountered more frequently, may reflect an initial impairment in coronary circulation and are independent predictors of coronary morbidity and mortality (Schillaci G et al, J Hypertens 2004; 22: 407-414). The aim of the present study is to assess the role of epicardial fat in the development of sub-clinical coronary abnormalities by evaluating the association of echocardiographic epicardial fat thickness with minor ST-T alterations in a population of untreated hypertensive adults.
Materials and Methods. 146 untreated hypertensive subjects (48±11 years, 61% males) underwent 12-lead ECG and 2D-echocardiography. Patients with prevalent cardiovascular disease, complete bundle branch block, poor-quality echocardiographic images or digoxin use were excluded. ST-T repolarization changes at ECG were defined according to Minnesota coding as the presence in any of leads I, II, aVL or V2-V6 of: (1) horizontal or downsloping ST-J depression <0.05 mV, (2) upsloping ST-J depression ≥0.1 mV, (3) flat, negative or diphasic (negative-positive type only) T wave, or (4) T-R wave amplitude ratio <1:20. Epicardial fat thickness was assessed by echocardiography by midventricular parasternal short-axis, as the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium, measured perpendicularly on the free wall of the right ventricle at end-systole (average of 3 cardiac cycles).
Results: Minor ST-T changes were observed in 9% of the sample. Subjects with ST-T changes were older (57±13 vs 47±10 years, p<0.001) and had more frequently echocardiographic left ventricular hypertrophy (56% vs 25%, p<0.05) than subjects with normal repolarization; sex distribution, blood pressure values, body mass index and waist circumference did not differ. Epicardial fat thickness was greater in the subjects with ST-T changes (5.5±2 vs 4.0±2 mm, p=0.018).
Epicardial fat thickness had a significant direct relation with body mass index (r=0.49) and waist circumference (r=0.43, both p<0.001), but not with age, sex, blood pressure or left ventricular mass. In a multivariable logistic regression analysis, epicardial fat thickness (odds ratio for each 1-SD increase, 2.07, 95% confidence interval 1.1-4.2), age (OR 2.68, 95% CI 1.1-6.7) and left ventricular mass (OR 1.78, 95% CI 1.0-3.5) were the only three independent predictors of minor repolarization changes.
Conclusions. Epicardial fat thickness at echocardiography is an independent predictor of prognostically adverse left ventricular repolarization changes in untreated subjects with uncomplicated hypertension. These findings are consistent with the suggested detrimental effects of epicardial fat on coronary circulation.

ROLE OF FIBRONECTIN EDA IN ATHEROSCLEROSIS

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Background. Fibronectins (FN) are multifunctional glycoprotein present in the plasma and in the extracellular matrix (ECM) of the tissues. The primary transcript undergoes alternative splicing to generate isoforms namely FN-EDA, FN-EDB and III-CS. FN-EDA is regulated spatially and temporarily 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. Previous evidences indicated that FN-EDA levels are increased during a series of pathophysiological condition including thrombosis, lung fibrosis, Inflammation.
Aim of this work was to investigate the role of FN-EDA in atherosclerosis in animal models.
Materials and Methods. Specific gene targeting allowed to generate mice that express fibronectin constitutively containing EDA or not. In order to investigate the role in atherosclerosis we generated a double knockout animal by crossing FN-EDA+/+, FN-EDA-/- with ApoE-/- .
Results. At two months of age animals were moved on high fat diet for 3 months. Preliminary analysis showed that atherosclerosis development is affected both in FN-EDA+/+; ApoE-/- and FN-EDA-/-ApoE-/- at the aortic root levels without a major effect on blood total cholesterol or triglyceride levels. Deposition of collagen and elastin showed that FN-EDA-/-;ApoE-/- animals present a more stable plaque compared to FN-EDA+/+ ;ApoE-/- and the controls. To further validate these data in a different model of atherosclerosis, we crossed FN-EDA-/- and FN-EDA+/+ with LDL-R-/- animals as well as generated a liver conditional FN-EDA-/- systemically FN-EDA+/+ ApoE-/-. Analysis of these new three mice models is in progress.
Conclusion. These data suggest a key role for FN-EDA in the development of atherosclerosis, further studies are warranted to elucidate the molecular mechanisms behind these effects.

TANGIER DISEASE: A PLASMA PROTEOME APPROACH

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Background. Tangier disease (TD) is a rare autosomal recessive disorder characterized by a deficiency or absence of high-density lipoprotein (HDL) caused by mutations in the adenotriphosphate-binding cassette transporter-1 gene (ABCA1). Mutations of ABCA1 lead to a defect in cellular cholesterol removal causing the deposition of cholesterol esters throughout the body. Individuals with TD are unable to eliminate cholesterol from cells which is then accumulated in the tonsils and other organs.
Methods. We enrolled a homozygous TD patient and the heterozygous father. Whole extracts were processed by a fractioning method on trypsin digested mixtures by means of C18 reverse-phase nano-HPLC chromatography. The column output were automatically mixed with a matrix, necessary for the next MALDI analysis, and spotted onto plate of mass spectrometer. Mass spectrometry analyses of peptide fragments were performed on MALDI TOF/TOF equipment.
Trypsin digestion produces a constant set of peptide fragments distinctive of each starting protein (finger print). Peptide ions were automatically processed by dedicated software, MASCOT, GPS Explorer, which provided us the identification of starting proteins. Peaks, indicative of different peptides, were also subjected to mass/mass analysis (TOF/TOF) by means of fragmentation in a collision chamber. Such second mass analysis provided aminoacid sequence of each peptide and the identification of possible post-translational modifications. Proteins identification were accounted providing the coverage of sequence by different peptides and the confidence limits of the attribution.
Results. We identified more than 2000 plasma proteins with a good sensitivity able to detect less represented proteins such as Prothrombin (1.2 pg/ml).
A number of differentially modulated proteins were observed and their statistical significance assessed. Clinical diagnostic assays, performed on some of the identified proteins, displayed an actual modulation of blood concentration between healthy and patients specimens confirming the differences observed in the proteomic comparative analysis
Conclusions. The high sensitivity of proteome analyses may help in the identification of abnormal metabolic pathways eventually activated in this disease.

ASSOCIATION BETWEEN THE ADHERENCE TO AHA STEP1 NUTRITION CRITERIA AND THE CARDIOMETABOLIC OUTCOME IN THE GENERAL POPULATION: A TWO YEAR FOLLOW-UP STUDY

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Introduction. The National Cholesterol Education Program and the American Heart Association consider a dietary therapy as a primary approach to prevent and treat hyperlipemia and hypertension. Mediterranean diet has been promoted as a model of healthy eating and widely recognized for favorable effects on lipid profile.
Objective. We investigated whether the adherence to dietary recommendations have any significant benefit on cardiovascular risk factors. Study Population: A cohort of 2141 of subjects attending our center were recruited by General Practitioners to participate in the PLIC Study.
Methods. Participants completed a week quantitative food questionnaire, which was analized on a subgroup of 338 subjects at enrolment (V1) and after two years of follow up (V2). Daily energy intake in Kcal, lipid, protein, carbohydrates in % of total energy, monounsatured (MUFA), saturated (SFA), polyunsaturaded fatty acids(PUFA) in g/die, cholesterol in mg/die was extrapolated from the food questionnaires. Cardiovascular risk (CVR) was calculated according Framingham algorithm.
Results. Subjects which adhered to AHA step 1 criteria showed a significant decrease in total cholesterol, 213.88±43.00 vs 220.19±39.3 mg/dL, LDL-cholesterol, 139.80±76.36 vs 142.75±35.60 mg/dL compared to subjects with an impaired dietary pattern while no differences were observed for HDL cholesterol.
Conclusions. Changes in dietary profile is associated with an improved cardio-metabolic profile and therefore remain one of the more favourable models in the primary prevention of cardiovascular disease.

IVABRADINE IMPROVES QUALITY OF LIFE IN SUBJECTS WITH CHRONIC ISCHEMIC DISEASE TREATED WITH BETA-BLOCKERS

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Aim. None of published studies evaluated quality of life (QoL) with a 36-items Short-Form (SF-36) questionnaire in subjects with chronic ischemic heart disease treated with Ivabradine (IVA). Subjects and methods. We evaluate if a 1 month treatment with IVA (5 mg bid) improves the QoL (assessed by SF-36 questionnaire) of patients with chronic cardiac ischemic heart disease taking β-blockers. SF-36 was tested in 138 patients (mean age 62 years) with chronic cardiac ischemic disease taking β-blockers and enrolled from a Cardiology Unit in the south

of Italy during 2009. Data were collected by an interview during a clinical visit both at prescription time (basal) and after 1 month of therapy with IVA. QoL life results after 1 month of therapy with IVA were compared with basal values.
Results. The IVA treatment was associated with an improvement of physical functioning (P=0.004), physical role functioning (P=0.02), role-emotional functioning (P=0.001), and psychic health scales P=0.001) in comparison with β-blockers treatment.
Conclusions. IVA treatment significantly improves the QoL in patients with chronic ischemic heart disease taking β-blockers.

LYCOPENE AND PRECLINICAL ATHEROSCLEROSIS

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Aim. Evidences from epidemiological and clinical studies suggest a possible correlation between serum antioxidant levels and the cardiovascular disease risk. High plasma concentrations of lycopene have been associated with reduced prevalence of cardiovascular disease. The aim of this study was to compare plasma concentrations of lycopene in subjects with or without ultrasonic evidence of asymptomatic carotid atherosclerosis. Subjects and methods. 120 subjects underwent physical examination, ultrasonic measurement of common carotid artery intima-media thickness, and serum laboratoristic samples.
Analysis of variance and logistic regression methods were used to determine whether differences existed between participants with or without evidence of carotid atherosclerosis.
Results. Of the 120 participants, 58 exhibited evidence of carotid atherosclerosis. Participants with ultrasonic evidence of carotid atherosclerosis exhibited significantly higher serum concentrations of total cholesterol, LDL-cholesterol and triglycerides. In contrast, participants with ultrasonic evidence of carotid atherosclerosis exhibited significantly lower plasma concentrations of lycopene.
Conclusions. These data suggest that higher serum levels of lycopene may play a protective role versus cardiovascular diseases, in particular carotid atherosclerosis.

CAROTENOIDS AND ASYMPTOMATIC ATHEROSCLEROSIS

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Aim. High plasma concentrations of lycopene and β-carotene have been associated with reduced prevalence of cardiovascular disease. The aim of this study was to compare plasma concentrations of these carotenoids in subjects with or without ultrasonic evidence of asymptomatic carotid atherosclerosis. Subjects and methods. 165 subjects underwent physical examination and ultrasonic measurement of common carotid artery intima-media thickness. Analysis of variance and logistic regression methods were used to determine whether differences existed between participants with or without ultrasonic evidence of asymptomatic carotid atherosclerosis.
Results. Of the 165 participants, 80 exhibited evidence of carotid atherosclerosis (carotid intima-media thickness >0.8 mm), while 85 did not (carotid intima-media thickness <0.8 mm). Participants with ultrasonic evidence of carotid atherosclerosis exhibited significantly greater body mass index, significantly higher serum concentrations of total cholesterol, LDL-associated cholesterol and triglycerides, and significantly higher plasma concentrations of uric acid, C-reactive protein and fibrinogen. In contrast, participants with ultrasonic evidence of carotid atherosclerosis exhibited significantly lower plasma concentrations of lycopene and β-carotene.
Conclusions. These results suggest that lycopene and β-carotene may play important roles in the delaying the development of the early asymptomatic stage of carotid atherosclerosis. Encouraging adequate intakes of antioxidant carotenoids may provide an important public health service.

THE EFFECT OF PHARMACOLOGICAL TREATMENT ON ASYMMETRIC DIMETHYLARGININE (ADMA) CONCENTRATION IN PATIENTS WITH ACUTE CONGESTIVE HEART FAILURE

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Aim. Asymmetric dimethylarginine (ADMA) plays a crucial role in the arginine-nitric oxide pathway. The purpose of this study was to investigate the effect of pharmacological treatment on asymmetrical dimethylarginine (ADMA) plasma levels in patients with acute congestive heart failure. Subjects and methods. Patients with symptomatic acute congestive heart failure (NYHA Class III-IV) HF and impaired left ventricular function (ejection fraction <40%) were included in the study. ADMA and SDMA concentrations were assessed before and after pharmacological treatment in 18 critically ill patients on the intensive care unit (ICU) by high performance liquid chromatography. All patients received a complete pharmacological treatment (diuretics, digoxin, ACE-inhibitors or angiotensin receptor blockers, β-blockers, and nitroglicerin) plasma concentration of ADMA and SDMA.
Results. ADMA plasma levels of critically ill patients were significantly higher after pharmacological treatment (0.64 μmol/L) respect baseline values (0.56 μmol/L) (p<0.001). Conclusions. In critically ill patients with acute congestive heart failure plasma ADMA levels were elevated and associated with the extent of multiple organ failure.

CAROTID ATHEROSCLEROSIS AND LIPID PROFILE IN A DIABETIC POPULATION: A RETROSPECTIVE EVALUATION

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Introduction. Type 2 diabetes mellitus is associated with a high risk of cardiovascular disease (CVD), particularly the atherosclerotic vascular disease. In diabetic patients the management of dyslipidemia, along with glycemic and blood pressure control, is important in the approach for prevention of CVD. Lipid abnormalities in diabetics consist of hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), and the presence of small dense low-density lipoprotein (LDL) particles. LDL cholesterol levels in patients with diabetes are similar to those found in non diabetic population. Therapies to reduce cardiovascular risk typically focus on statins; however, clinical and epidemiologic data showed that, even when LDL-C is managed successfully, the residual cardiovascular risk is associated with low HDL-C levels and elevated triglycerides (TG) levels. The aim of our study was to evaluate the prevalence of "on target" subjects (with LDL-C <100 mg/dl), the prevalence of classical risk factors associated with vascular damage, and the prevalence of carotid atherosclerosis, in a population affected by type 2 diabetes mellitus who have never received lipid lowering drugs.
Materials and Methods. We analyzed, from the archive of the Diabetes Centre of Internal Medicine Department of the University of Messina, data of 400 patients (198 male, 202 female), who were never treated with any lipid lowering drugs, from 2000 up to 2009. We evaluated: anthropometric parameters, lipid profile, transaminase, glucose and HbA1c plasma levels, and carotid atherosclerosis by ecocolor-Doppler.
Results. We found that only 17% of diabetics showed LDL-C to target (<100 mg/dl). Low HDL-C levels were detected in 70 males (35%, HDL-C ≤40 mg/dl) and 104 females (51%, HDL-C ≤50 mg/dl). In LDL-C "on target" group the prevalence of low HDL-C levels was similar to that found in the entire sample (M=31%, F=51%). Moreover systolic blood pressure (SBP) ($p<0.05$), total cholesterol ($p<0.001$), TG ($p<0.05$), and non-HDL-C ($p<0.001$) were significantly lower in this group respect to patients with baseline LDL-C >100 mg/dl. In LDL-C "on target" group the prevalence of carotid atherosclerosis was 33%, while it was 43% in LDL-C >100 mg/dl group. On the other hand, 40% of patients with atherosclerosis showed low HDL-C levels (M=34%, F=49%) We observed a significant correlation between carotid atherosclerosis and age ($p<0.001$), gender ($p<0.05$), SBP ($p<0.001$), HDL-C ($p<0.05$) and HbA1c ($p<0.05$). The multiple regression analysis showed that in this population, the IMT values are affected by age, and by HDL-C values ($p=0.001$).
Conclusions. This report confirms that, in diabetic patients, who were never treated with lipid lowering drugs, the main lipid factor able to affect the progression of atherosclerotic vascular disease is HDL-C, more than LDL-C levels. Although it was widely shown that the lipid lowering strategies, particularly with statins, are able to improve the cardiovascular risk profile, it is true that the LDL-C levels are not the only objective to be achieved.
The high prevalence of low HDL-C levels in diabetes, besides the hypertriglyceridemia, presents further targets for the development of new cardioprotective therapies.

PREDICTION OF VASCULAR EVENTS IN SUBJECTS WITH SUBCLINICAL ATHEROSCLEROSIS AND THE METABOLIC SYNDROME: THE ROLE OF MARKERS OF INFLAMMATION

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Background. The presence of the metabolic syndrome (MS) increases cardiovascular morbidity and mortality but few data is available on the outcome in subjects with the MS and subclinical atherosclerosis.
Aim. We aimed to assess cardio- and cerebro-vascular events in subjects with MS and subclinical atherosclerosis. Methods: We followed-up for five years 339 subjects with asymptomatic carotid intima-media thickness >0.9 mm, of whom 130 had the MS, evaluating at baseline traditional cardiovascular risk factors (including male gender, older age, obesity, hypertension, diabetes, smoking, family history of cardiovascular diseases, dyslipidemia) and plasma levels of C-reactive protein and fibrinogen.
Results. Cardio- and cerebro-vascular events were registered in the 29% of subjects with the MS and in the 20% of those without it and the presence of more criteria for the diagnosis of the MS was significantly associated with vascular morbidity and mortality: with transient ischemic attack ($p<0.0001$), angina ($p=0.0022$), cardio- and cerebro-vascular death ($p=0.0019$) and the presence of any clinical event ($p=0.0003$). Further, we used logistic regression analysis to search for possible independent associations of any parameter evaluated at baseline with the occurrence of clinical events and we found a predictive role for elevated markers of inflammation (OR 3.8, 95% CI 2.6-12.5, $p=0.0022$), elevated fasting glucose (OR 2.1, 95% CI 1.2-4.0, $p=0.0134$) and elevated triglycerides (OR 1.4, 95% CI 1.1-2.9, $p=0.0351$).
Conclusions. These findings confirm a worst vascular outcome in subjects with more criteria for the diagnosis of the MS and further suggest the need of future research to understand the combined role of inflammation and the MS in the progression from subclinical to clinical atherosclerosis.

THROMBOPATH® DETERMINATION DURING 1 YEAR-FOLLOW-UP IN ACUTE CORONARY SYNDROMES

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Acute coronary syndrome (ACS) is a state induced by thrombosis consequent to unstable atherosclerotic plaque rupture. The plaques' procoagulant content triggers platelet and coagulation activation. After acute cardiovascular events occurrence, a hypercoagulable state has been documented, but few data on blood clotting activation durability are available.
Thrombopath® (ThP, Instrumentation Laboratory) is a global

assay sensitive to prothrombotic factors and activated protein C. We aimed to evaluate the balance between anticoagulant and procoagulant factors, by using the ThP, during a follow-up of 12 months after an acute event in 115 (87 males/28 females) ACS patients undergoing percutaneous coronary intervention (PCI) with stent implantation on dual antiplatelet treatment.
ThP analysis was performed at the time of acute coronary events (TØ), after 1 (T1), 6 (T2) and 12 (T3) months. Altered ThP cut-off value was set at 77.31% protac-induced coagulation inhibition (PICI% mean values in 150 controls-2SD).
Baseline values (TØ) of ThP were significantly lower in ACS patients than in controls [78.84(40.82-94.11)% vs 87.48(72.50-97.65)%, p<0.0001].
PICI values significantly increased during the 12 month follow-up (p<0.0001). At T1 PICI values were slightly but not significantly higher than those observed at TØ [80.62(51.00-91.02)% vs 78.84(40.82-94.11)%]. After 6 and 12 months of follow-up a marked increased with respect to baseline PICI values was observed [T2: 82.42(53.96-91.39)%; T3: 83.32(67.07-91.77)%, p<0.0001 vs TØ].
At TØ altered ThP values were found in 46.1% of ACS patients. Similar figure was found at T1: altered ThP values were present in 41.7% of patients. Abnormal ThP values significantly (p<0.01) decreased to 25.2% at T2 and 21.7% at T3.
This study demonstrates that a marked unbalance of coagulation cascade persists 1 month after the acute coronary event. In spite of the progressive increase in ThP values during the follow-up a hypercoagulable state is yet present 6 and 12 months after the vascular events.

PERFORMANCE EVALUATION OF A NEW RAPID LATEX IMMUNOASSAY FOR THE DETECTION OF ANTI PF4/HEPARIN COMPLEX ANTIBODIES

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Heparin-induced thrombocytopenia (HIT) is characterized by platelet-activating antibodies directed against complexes of heparin with platelet factor 4 (anti-PF4/Hep). The antigen-based immunoassays are characterized by high sensitivity and low specificity. It is now available an automated latex enhanced immunoassay for the determination of total antibodies anti-PF4/Hep.
This study was aimed to evaluate the performance of a new rapid immunoassayin detecting anti-PF4/Hep antibodies. In57 patients investigated for suspected HIT we performed: 1-ELISA assay (GTI Diagnostic), 2-rapid automated latex enhanced immunoassay (Instrumentation Laboratory) and 3-functional assay heparin-induced platelet aggregation (HIPA).
23/57(40.4%) patients had a positive test with ELISA method (≥0.4 O.D.) and 15/57(26.3%)with latex immunoassay (≥0.7 U/ml). Ten patients were positive at both tests, and 18 patients haddiscordant results: 5 patients hada positive latex immunoassay but negative ELISA and 13 had positive ELISA test but negative latex immunoassay. 2/23 patients, positive for ELISA test, had a low pretest probability (4T-score 0-3), 14/23 had a moderate pretest probability (4T-score 4-5) and 7had a high pretest probability (4T-score 6-8). Among patients positive for the latex immunoassay 2/15 patients had a low pretest probability, 9/15 had a moderate and 4had a high pretest probability.
By using HIPA test as gold standard, ROC curve analysis showed that both tests had a 100% sensitivity, whereas specificity was 63%(50-76%, C.I. 95%) for the ELISA method and 78%(67-89%, C.I. 95%)for the latex immunoassay. The optimal cut-off values for identifying patients positive at HIPA test was 2.0 O.D. for the ELISA test and 2.7 U/ml for the latex immunoassay test. Our results demonstrate high sensitivity of both ELISA and latex immunoassay test, with a higher specificity for the rapid latex immunoassay, suggesting that this rapid and easy to perform assay may be a useful tool for laboratories to detect anti-PF4/Hep antibodies.

A FIRST COMPARATIVE STUDY ON TWO CELL CULTURE TECHNIQUES - STIMULATED T CELLS AND CONTINUOUS LYMPHOBLASTOID CELL LINES- IN THE DETECTION OF LDL RECEPTOR RESIDUAL ACTIVITY VERSUS MOLECULAR GENETIC ANALYSIS

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Introduction. In Familial Hypercholesterolemia (FH) the impairment of LDL-R activity detected by flow cytometry (FCM) could allow to identify patients bearing mutations into the LDL-R gene. The LDL-R activity can be studied either by using continuous lymphoblastoid cell lines or mitogen stimulated T cells obtained from peripheral blood samples. Therefore, we matched these two culture systems in order to: 1) identify the best way to detect LDL-R activity; 2) determine the reference range for controls; 3) examine LDL-R residual activity in presence of mutations.
Materials and Methods. Lymphoblastoid cell lines were generated by the exposition of mononuclear cells to EBV enriched medium. T-cells were stimulated for 48h in IMDM supplemented with 10ng/mL Phorbol Myristate Acetate, 1uM Ionomycin and 10% human lypoprotein deprived serum in order to enhance LDL-R. FCM functional assay was performed by incubation with fluorescent LDL (Dil-LDL) for 2h at 37°C and analyzed by FACSCanto II.
Results. We determined the FCM reference range for LDL-R activity in both lymphoblastoid cell lines and stimulated T cells obtained from healthy donors. We also determined LDL-R residual activity for FH patients using the two methods. Data obtained using lymphoblastoid cell lines or stimulated T cells give comparable results. Reduced residual LDL-R activities were in agreement with the presence of LDL-R mutations.
Conclusions. We compare for the first time two cell culture techniques useful to detect LDL-R residual activity in presence of the most frequent mutations in Southern Italy FH patients. In

our view the use of stimulated T cells may be helpful for the characterization of LDL-R activity for the following reasons:
1) comparability of the results with those obtained through lymphoblastoid cell lines;
2) more rapid generation of stimulated T cells respect to lymphoblastoid cell lines;
3) the possibility of using stimulated T cells also in class 1 biosafety level laboratories.

SIMVASTATIN IMPROVES IN VITRO PLATELET SENSITIVITY TO ASPIRIN IN PATIENTS WITH PRIMARY HYPERCHOLESTEROLEMIA: RELATIONSHIPS WITH LIPIDS AND CYTOKINES

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Background and Aims. Patients with primary hypercholesterolaemia should be treated by aspirin to reduce their elevated cardiovascular risk. Since hypercholesterolemia can induce aspirin resistance, we investigated in these patients the effects of simvastatin on in vitro platelet sensitivity to aspirin.
Materials and Methods. 14 patients (M/F 7/7; age: 56.0±2.9 years; BMI: 26.6±1.2 kg/m^2) affected by primary hypercholesterolaemia, without previous cardiovascular events, were enrolled after two months of lifestyle intervention and before prescription of hypolipidaemic and anti-aggregating drugs. At baseline and after two months of statin therapy (simvastatin, 40 mg/day) we measured: a)in platelet-rich plasma (PRP), responses to 1 mmol/l Na Arachidonate (NaAA), 20 micromol/l ADP and 4 mg/l Collagen (Born's method) and the inhibition of platelet aggregation and of thromboxane B2 (TXB2) synthesis (EIA) exerted by lysine acetylsalicylate (LAS, 1-300 micromol/l) in response to agonists; b)in serum, lipid parameters, circulating pro- and anti-inflammatory cytokines and markers of in vivo platelet activation.
Results. After statin therapy, we observed: a)reduction of total cholesterol by 35.1±2.9% (p<0.0001), of LDL-cholesterol by 46.1±3.8% (p<0.0001), of ApoB by 38.1±3.4% (p<0.0001), of triglycerides by 13.3±11.6% (p<0.05), with unchanged HDL cholesterol; b)reduction of platelet responses to agonists, being Maximal Aggregation (MA) 75.9±1.9% vs 90.7±3.9% (p<0.002) with NaAA; 71.7±1.2% vs 90.5±3.0% (p<0.0001) with ADP and 71.6±2.2% vs 82.7±2.7% (p<0.016) with Collagen; c)reduction of TXB2 production (ng/ml) in response to agonists: 476.4±88.1 vs 775.4±76.3 (p<0.009) with NaAA; 14.0±2.1 vs 24.5±2.1 (p<0.0001) with ADP, 19.4±2.0 vs 42.7±8.0 (p<0.015) with Collagen; d) increase of platelet sensitivity to ASA, being LAS IC-50 (micromol/l) for NaAA-induced aggregation 10.6±1.8 vs 37.3±3.7 (p<0.0001), LAS IC-50 for TXB2 synthesis induced by NaAA 9.0±3.0 vs 23.5±4.1 (p<0.009), LAS IC-50 for TXB2 synthesis induced by collagen 12.8±2.6 vs 28.4±4.7 (p<0.013); e)reduction of pro-atherogenic and pro-inflammatory parameters: hsCRP (0.33±0.08 vs 0.64±0.11 mg/dl; p<0.012), sCD-40L (8.32±0.97 vs 12.95±1.05 ng/ml; p<0.013), sE-selectin (28.54±3.43 vs 41.90±4.24 ng/ml; p<0.039), IL-1beta (0.63±0.12 vs 1.27±0.09 pg/ml; p<0.0001), IL-8 (13.2±2.0 vs 19.55±1.29 pg/ml; p<0.009), IFN-gamma (18.65±2.14 vs 31.43±4.65 pg/ml; p<0.05), VEGF (93.55±11.66 vs 59.14±12.80 pg/ml; p<0.05) and increase of anti-atherogenic and anti-inflammatory parameters: IL-1ra (67.74±7.75 vs 44.74±6.53 pg/ml; p<0.04), IL-4 (1.38±0.08 vs 1.04±0.08 pg/ml; p<0.008), IL-10 (1.94±0.11 vs 1.18±0.15 pg/ml; p<0.001) and sRAGEs (1214.07±99.04 vs 832.29±64.83 pg/ml; p<0.0001). ASA IC-50 for NaAA-induced aggregation correlated positively with Total Cholesterol (r=0.749, p<0.0001), LDL-cholesterol (r=0.771, p<0.0001), sCD-40L (r=0.518, p<0.007) and negatively with IL-10 (r=-0.579, p<0.002), IL-4 (r=-0.513, p<0.007) and sRAGE (r=-0.429, p<0.05)
Conclusion. Treatment with simvastatin in patients with primary hypercholesterolaemia improves in vitro platelet sensitivity to aspirin, which correlates with Total and LDL-cholesterol and with some cytokines and inflammation mediators.

P2X7 RECEPTORS IN HUMAN ADIPOCYTES MODULATE SOME INFLAMMATORY RESPONSES IN SUBJECTS WITH METABOLIC SYNDROME

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Introduction. No information is available on the presence of P2X7 receptor in human adipocytes and its potential involvement in the chronic inflammation associated with metabolic syndrome (MS).
Methods. Adipocytes were isolated from samples of visceral (VAT) and subcutaneous (SAT) adipose tissue of 40 patients with MS and 20 controls (CTL). We measured adipocyte gene expression of TNFα,IL-6 and PAI-1 (by realtime-PCR) and their plasma concentrations (ELISA), and P2X7 gene and protein expression (realtime-PCR, Western blot and immunofluorescence). P2X7 functional activity was estimated by measuring the effect of BzATP (agonist) and KN62 (blocker) on intracellular calcium fluxes ([Ca2+]i, by fluorimetry, and adipocytokine release, by ELISA.
Results. In VAT, TNFα, IL-6 and PAI-1 were more expressed in MS than in CTL (T/R ratio: 3.29±1.47 vs 1.79±1.19; 8.99±4.24 vs 5.65±4.03; 6.06±2.32 vs 2.91±0.34,p =0.005-0.0001). These differences were confirmed in SAT for IL-6 (3.56±1.56 vs 1.98±1.49, p=0.0004) and PAI-1 (3.87±1.87 vs 2.25±1.16, p=0.008), but not for TNFα. Plasma IL-6, PAI-1 and TNFα levels were higher in MS (IL-6: 2.81±1.55 vs 4.32±2.67 pg/ml, p=0.002; PAI-1: 32.08±11.21 vs 42.26±11.6 ng/ml, p=0.002; TNFα: 3.22±1.26 vs 2.51±0.88 pg/ml, p<0.05).
P2X7 mRNA, found both in VAT and SAT, was more abundant in MS than in CTL (T/R ratio: 2.13±0.68 vs 1.56±0.49 in VAT,p=0.0013 and 1.76±0.54 vs 1.46±0.41 in SAT,p=0.03). Protein expression confirmed this observation, with the typical "ring-like" arrangement of P2X7 receptor at the plasma membrane. BzATP 0.5 mM raised [Ca2+] i in VAT and SAT, without differences between MS and CTL (VAT: +128 vs +98%, p=ns; SAT: +107 vs +110%, p=ns). In both MS and CLT cells BzATP induced IL-6 and TNFα release, partially inhibited by KN62 (VAT: IL-6 from 141±33 to 308±28 and 233±33 with KN62 in CTL; from163±27 to 318±64 and 254±59 pg/ml/mg tissue with KN62

in MS,p<0.0001; TNFα from 2.3±0.7 to 3.7±0.8 and 3.1±0.9 with KN62 in CTL; from 2.3±0.6 to 4.0±0.8 and 3.2±0.9 pg/ml/mg tissue with KN62 in MS,p<0.0001). The effect on PAI-1 release was less pronounced.

Conclusion. Human adipocytes express functionally active P2X7 receptors, which modulate the release of some inflammatory cytokines. Adipocytes from MS patients show an enhanced P2X7 receptor expression, which might contribute to the subclinical inflammatory status characterizing these patients.

SECONDARY PREVENTION OF MYOCARDIAL INFARCTION AND LONG-TERM ADHERENCE TO STATIN THERAPY: DATA FROM THE ALARM2 INVESTIGATION

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Prescription and adherence to statin treatment hold distinct but complementary roles in the beneficial effects due to preventive interventions. We evaluated these parameters in subjects affected by myocardial infarction (MI), correlating such data to re-infarction and total mortality rate. ALARM2 (Adherence to Long-term therapies: Assessment and Real practice Management -2) is a prospective investigation carried out in the province of Ferrara (Italy), aiming at evaluating efficacy and costs of HMG-CoA reductase inhibitor utilization.

The analysis of clinical-administrative and prescriptive databases of the whole resident population allowed the identification of 1529 subjects (934 men and 595 women) aged more than 18 (72.6±13.0 ys., mean±S.D.), discharged from hospital wards with a diagnosis of MI in three consecutive periods of one year each. Statin prescription and treatment adherence (estimated by the proportion of days covered by treatment) were evaluated in the following 2 years, together with total mortality and re-infarction rates.

For persistent statin users, we categorized patients as adherent if the medication possession ratio was >/=80%. Statin-treated subjects (STS), compared to not-treated ones (NTS), were younger, more frequently males and assuming other cardiovascular drugs. These same characteristics did not discriminate subjects whose adherence in treatment was satisfactory (good adherent subjects, GAS) or insufficient (bad adherent subjects, BAS). After correcting for several confounders (age, gender, cohort, drugs, comorbidity) total mortality hazard ratio (HR) was 0.438 in BAS and 0.194 in GAS compared to NTS (p<0.001), while HR for reinfarction was 0.396 in BAS and 0.374 in GAS compared to untreated subjects (p<0.01); data were replicated after dichotomizing subjects according to age (less than / more than 80 years). This analysis, carried on in non-selected MI subjects, showed better survival and less recurrent ischemic heart disease events related to statin use: to optimize patient outcomes, clinicians should consider clinical and social factors that impact adherence.

ARTERIAL INTIMA-MEDIA THICKNESS, ENDOTHELIAL FUNCTION AND LIPOPROTEIN SUBFRACTIONATION IN SUBJECTS WITH VERY-HIGH HDL SERUM LEVELS: THE HALA STUDY

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Prospective observational studies show that there is a consistent inverse relationship between serum HDL-cholesterol (HDLc) levels in the general population and risk of cardiovascular events. HDL athero-protection has been related to reverse cholesterol transport (RCT) promotion, but also to a favorable role on endothelial function, low inflammation and thrombosis prevention. However little evidence support HDLc benefits beyonds the epidemiological boundaries of 30-75 mg/dl: data concerning risk profile in subject with very high HDLc levels (hyperalphalipoproteinemia, HAL) are limited and controversial while drug treatment aimed at highly increasing HDLc have not proved helpful. The HALA (HyperAlphaLipoproteinemia and Atherosclerosis) study is a case-control investigation planning to explore the genetic background of very high serum HDLc (>/= 3 S.D. from mean HDLc concentration in normal population), and determine the relationships between this condition and lipoprotein particle distribution, inflammatory parameters and subclinical vascular disease (by considering different markers of arterial morphology and function - ankle-brachial index, ABI; carotid intima-media thickness, IMT; brachial-artery flow mediated dilation, FMD).

Twenty subjects (18 F, 2 M; aged 52.4±7.7 ys) with HDLc suggestive of genetic HAL (HDLc >85 mg/dl; absence of significant heart, kidney and thyroid diseases; not taking drug known to interphere with HDL metabolism) and 20 healthy age, sex and LDL-cholesterol (LDLc) matched-controls (18F, 2M; aged 51.3±8.0 ys) have so far been evaluated. Fasting HDLc levels were 97±13 mg/dl in HAL-subjects and 57±12 mg/dl in controls (p<0.001), with LDLc levels of 143±35 and 132±35 mg/dl (p=n.s.) respectively. ABI, common carotid-IMT and plaque number (both in carotid and femoral arteries) did not differ in cases and controls; HAL subjects disclosed lower Resistance Index at doppler flow velocity analysis of common carotid artery, and higher FMD (borderline significance) of shorter duration compared to the control group. In conclusion this echo-color-doppler preliminary vascular investigation does not evidence anatomical markers of subclinical atherosclerosis differentiating middle-aged HAL-subjects from controls. On the other hand HAL subjects seem to show functional performance on ischemia-induced vasodilation suggesting arterial protection.

ANGIOTENSIN CONVERTING ENZYME POLYMORPHISM IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background. LDL apheresis is indicated in Homozygous Familial Hypercholesterolemia patients and in drug refractory Heterozygous Familial Hypercholesterolemia with coronary heart disease. This therapy usually is well tolerated but can be complicated by bradykinin release, directly activated by LDL apheresis systems. The bradykinin release may develop anaphylactoid reactions with different severity. Angiotensin Converting Enzyme (ACE) I/D polymorphism, possibly linked to the bradykinin system activation, was investigated in a group of refractory FH patients on LDL apheresis treatment.
Method. The ACE gene I/D polymorphism was detected by PCR in a group of 20 patients with Familial Hypercholesterolemia complicated by coronary heart disease and regularly treated by LDL apheresis. The ACE gene I/D polymorphism of this patients was compared with that of a control group of 138 healthy subjects.
Results. The DD genotype frequency of ACE gene I/D polymorphism, compared to genotypes ID and II, was found to be significantly higher ($p = 0.04$) in the group of patients respect to the healthy subjects.
Conclusion. The ACE gene I/D polymorphism analysis in familial hypercholesterolemia may be useful to identify subjects more prone to develop coronary heart disease. Key words: LDL apheresis, angiotensin converting enzyme, gene polymorphism, dyslipidemia.

A CASE OF LCAT DEFICIENCY SECONDARY TO MALIGNANT NON-HODGKIN LYMPHOMA

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Background. Primary LCAT Deficiency is a rare genetic disorder caused by a mutation in the LCAT gene and characterized by severe hypoalphalipoproteinemia. Here we describe a case of secondary LCAT deficiency in a woman affected by malignant lymphoma.
Methods and Results. In May 2007, a 71-year-old woman presented to our lipid clinic with greatly reduced plasma high density lipoprotein-cholesterol (HDL-C) (5 mg/dl) and reduced total cholesterol (111 mg/dl) levels. Further analyses showed that all the circulating cholesterol wasn't esterified; cholesterol esterification rate (CER) was null (n.v. 30-60 nmol/ml/h) and LCAT activity was reduced (21.0 nmol/ml/h, n.v. 25-55 nmol/ml/h), despite normal plasma LCAT concentration (3.5 µg/ml, n.v. 3.1-6.7 µg/ml). Genetic analysis showed no mutation in the LCAT gene. Interestingly, previous analyses of the subject revealed a normal lipid profile. Afterwards, the patient was diagnosed malignant non-Hodgkin lymphoma. We thus hypothesized the presence of an LCAT inhibitor in the serum of the patient. To prove our hypothesis, we run an inhibition test by measuring CER after mixing (1:1, v:v) the serum of the patient with a control serum; indeed, CER was undetectable in the mixed sample. In November 2007, after cancer remission, the patient returned to our evaluation; the lipid profile was normal and both CER and LCAT activity were in the normal range (42.6 and 25.0 nmol/ml/h). We thus repeated the inhibition test, which showed no CER inhibition by the patient serum. In searching of the inhibitor, we used a gel shift analysis where the Antibody- Antigen complex was detected by western blotting using anti-LCAT and anti-human IgG antibodies.
Conclusion. We showed for the first time a case of complete LCAT deficiency secondary to malignant non-Hodgkin lymphoma and due to an antibody able to completely inactivate the LCAT enzyme.

HIGH PREVALENCE OF FH PADOVA 1 MUTATION IN A COHORT OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA ATTENDING THE LIPID CLINIC OF PADOVA

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Genetic heterogeneity of familial hypercholesterolemia (FH) represents a limit to molecular diagnosis. 13/133 LDLR mutations described in Italy are more frequent, D200G (FH Padova1) is the most represented (6,3%).
We investigated FH Padova1 prevalence in Veneto region seeking D200G mutation via PCR amplification in 63 individuals with strong FH clinical features; in negative cases, LDLR sequencing and rearrangements analysis were performed. LDLR mutations were identified in 57/63 individuals (90%). FH Padova1 was present in 34 subjects (54%), other mutations in 23 subjects (36%), so D200G showed an eight fold higher prevalence compared to the whole country. Cascade screening in relatives identified 47 FH subjects.
So, our case series includes 104 FH individuals with known genetic defect (age range 9-72 years), characterized by severe hypercholesterolemia (mean TC 374, LDLC 297, HDLC 53, TG 117 mg/dL) and high prevalence of early-onset coronary heart disease (CAD ≤55 in males and ≤65 in females in 28 subjects, 25%; mean age at onset 44 years).
The classic paradigm of FH is that CAD is mainly due to LDL atherogenicity, while the role of other cardiovascular risk factor is controversial. Our data suggest that these risk factors have a role in premature CAD development. To confirm this we compared 14 subjects with very early-onset CAD (≤40 years) and 17 subjects that reached age ≤60 years without cardiovascular events. These patients didn't differ significantly in terms of LDL, while we found significantly different HDL levels (43 vs 59 mg/dL $p<0,05$), BMI (29,7 vs 25,5 $p<0,05$) and percentage of smokers (92% vs 29%). Our study, although with a retrospective design, confirmed the role of some classical risk factors in premature CAD of FH. So, also in subjects with a monogenic disease, a multifactorial preventive approach is needed at the earliest age.

ANALYSIS OF LIPID LOWERING TREATMENT IN PATIENTS AT HIGH CARDIOVASCULAR RISK DISCHARGED FROM S.ORSOLA-MALPIGHI HOSPITAL, BOLOGNA

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Atherosclerosis is the leading cause of cardiovascular morbidity and mortality around the globe. One of its principal risk factor is dyslipidemia. Aim of our study was to determine the accuracy of dyslipidemia treatment in high-risk patients (recommended LDL cholesterol<100 mg/dl). We considered 25947 patients aged 40-70 years hospitalized at S. Orsola-Malpighi Hospital, Bologna during the whole 2008. We selected 4792 patients discharged from the Cardiology and Internal Medicine Departments: 1686 had established arterial disease (coronary artery disease, cerebrovascular disease, peripheral arterial disease) or diabetes; only 1246 of them had a complete lipid profile in their medical record. Then, we excluded patients with creatine kinase or transaminases alterations because of the impossibility to start a statin therapy.
The resulting population was of 999 subjects. Total cholesterol, HDL-cholesterol and triglycerides were measured on blood samples. LDL-cholesterol value was obtained by the Friedwald formula. Statins use was evaluated through medical records, comparing admission and discharge. Considering all 460 subjects with LDL-cholesterol levels >100 mg/dl, statins treatment increased from 25,54% at admission to 61,69% at discharge: however a large amount of patients who deserved a pharmacological therapy was not treated. In the subgroup with LDL-cholesterol levels >130 mg/dl (158 subjects) statins use increased from 20,6 to 66,33%. However 33,77% of subjects didn't receive a cholesterol lowering drug yet: therefore a lacking attitude to prescribe statins emerged also in these very high risk patients. In addition, we observed that only 10% of patients with LDL-cholesterol levels >100 mg/dl who were already under statins treatment received a higher dosage of the drug. Our data show that dyslipidemia is generally undertreated in high risk patients, despite the fact that hospitalization brings them in contact with specialized physicians.

ELECTROCARDIOGRAPHIC CHARACTERIZATION OF ISOLATED NON-ACUTE APICAL MYOCARDIAL INFARCTION

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Background. Electrocardiographic (ECG) criteria for the identification of the site and extension of myocardial infarction (MI) are well established, but identification of isolated apical MI is controversial and lacks of accuracy.

Methods. fourty-seven Tc-99m sestamibi myocardial scintigraphies showing isolated apical infarction [37 males (78.7%)] were obtained and then divided according to the site of basal non-reversible perfusion defect: 14 were isolated apical (29.8%), 6 inferior-apical (12.8%) and 27 anterior-apical (57.4%) MI. ECG were analysed according to the presence of reduced R wave amplitude in the precordial leads (21 pts, 44.7%), Q waves in the anterior leads (23 pts, 48.9%), in one of (2 pts, 4.3%) or all (9 pts, 19.1%) inferior leads, in one of (2 pts, 4.3%) or all (8 pts, 17.0%) lateral leads, in one of (5 pts, 10.6%) or all (4 pts, 8.5%) the upper lateral leads (DI and aVL) and the presence of tall R waves in the right precordial leads (5 pts, 10.6%).
Results. Q waves in the anterior leads were more significantly associated with anterior-apical MI (21/27 pts, chi-square 22.99, p=0.00001), whereas none of those with isolated apical MI showed Q waves in the anterior leads. Tall R waves in the right precordial leads were more frequently associated with inferior-apical MI (3/6 pts, chi-square 11.32, p=0.003) while isolated apical infarction showed a strong association with the presence of reduced R wave amplitude in the precordial leads (13/14 pts, chi-square 18.72, p=0.00008). Among pts with reduced R wave amplitude in the precordial leads, 6 pts had also Q waves in one of or all the anterior leads and/or in one of or all the inferior leads. But if we considered only reduced R wave amplitude in the precordial leads without Q waves in any other lead, the association with isolated apical MI became stronger (12/14 pts, chi-square 29.82, p=0.0000003).
Conclusions. previous isolated apical MI is often associated with reduced R wave amplitude in the precordial ECG leads, rather than with Q waves.

THE CLASS II PHOSPHOINOSITIDE 3-KINASE ISOFORM β; REGULATES PLATELET FUNCTION AND ARTERIAL THROMBOSIS IN MICE

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Phosphoinositide 3-kinases (PI3Ks) are crucial components of many signaling pathways involved in platelet activation. A key role is played by the class I enzymes and especially by the p110β isoform that regulates sustained αIIbβ3 integrin activation and modulates the outside-in signaling properties of activated αIIbβ3 integrin. By contrast very little is known about the role of the class II PI3Ks on platelet function, though the absence of phosphatidylinositol-3,4,5 triphosphate, the main lipid product of class I PI3Ks, during late phases of platelets aggregation and the activation of the class II PI3K isoform β (PI3K C2β;) via activated αIIbβ3 integrin suggest the involvement of these enzymes in the regulation of platelet functional responses. Using a PI3K C2β knockout mouse model we showed for the first time that PI3K C2β activity is required for normal collagen and thrombin induced platelets aggregation. Interestingly absence of PI3K

C2β does not affect inside-out signaling, indeed the activation of intracellular kinases, the α-granules release and the activation of αIIbβ3 integrin are similar in PI3K C2β deficient and wild type platelets.
On the other hand analysis of thromboxane A2 (TxA2) synthesis, show that agonists induced TxA2 production is significantly impaired in platelets from PI3K C2β knockout mice; the unaltered abundance and distribution within the different membrane phospholipids of arachidonic acid (AA) and the similar aggregation in response to AA and U46619, a synthetic TxA2/PGH2 receptor agonist, suggest that PI3K C2β regulates the activation of phospholipase A2 and AA mobilization, possibly downstream activated αIIbβ3 integrin. In vivo studies show that PI3K C2β knockout mice have a normal bleeding time but thrombus formation after FeCl3 injury of the carotid artery is significantly delayed in these mice. Taken together our data indicate the PI3K C2β enzyme as an interesting new target for antithrombotic drugs development.

USE OF LUNAR DXA TO MEASURE THEBODY FAT MASSON THE GENERAL POPULATION: RESULTS OF THE PLIC STUDY

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The relationship between fat, metabolic syndrome and cardiovascular risk is well proved. The body composition measurement through the dual-energy x-ray (DXA) absorptiometry, is able to visualize the body fat distribution, the lean and bone mass in different body size.
The aim of this study has been to estimate the use of Lunar DXA for the fat,lean and bone mass characterization, on the general population in relationship with the metabolic syndrome parameters. Through the PLIC Study, 950 subjects have been analysed (391 women and 559 men).
Statistical analysis have showed a significant correlation between the percentage of the fat and the body mass index (BMI). Significant differences have been observed in the android and gynoid body fat distribution between men and women.Further statistical analysis will examine the obtained data.
Following those results Lunar DXA allows, in a non-invasive way, to value the fat body mass distribution in the different body size, allowing a detailed and better assessment of this parameter, so important in the diagnosis of the metabolic syndrome and the cardiometabolic risk.

OBESITY, DIABETES MELLITUS AND METABOLIC SYNDROME IN THE ITALIAN POPULATION: THE CHECK STUDY

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The aim of this study was to describe subgroups of the CHECK cohort characterised by the presence of obesity, diabetes mellitus type 2 (DM2) and/or metabolic syndrome (MetS).
CHECK is a randomized Italian epidemiological study on 5846 subjects, between 40 and 79 years. For the present analysis the following medical conditions were considered: obesity (BMI ≥30mg/kg2), DM2 (fasting glucose ≥126 mg/dL, previous diagnosis and/or hypoglycemic therapy), and MetS-noDM2 (according to ATP III criteria; diabetic subjects were excluded). Global CV risk (% in 10 years; GCVR) was assessed according to the CUORE score. 5685 subjects had all the data for evaluation. 3612 (63.5%) did not have any of above mentioned conditions; 176 (3.1%) had all clinical conditions. Prevalences were 17.3%, 15.0% and 12.7% for obesity, MetS-noDM2 and DM2, respectively. Male represented 45.6%, 44.3% and 59.8%, while adults 69.2%, 67.7% and 53.9% of subjects with obesity, MetS-noDM2 and DM2, respectively. AMI and PVD prevalences increased from subjects with obesity (3.5% and 1.8%, respectively), to those with MetS-noDM2 (4.6% and 1.9%, respectively), to those with DM2 (6.5% and 4.4%, respectively). Mean (±SD) GCVR in the CHECK cohort was 5.21 (±5.93) and was significantly higher only in diabetic subjects [12.18 (9.78)], but its distribution among standard risk classed was very different in the three groups with 4.9%, 2.9% and 18.4% of subjects at high risk (RCVG ≥20%). C-reactive protein, a marker of inflammation, showed a trend towards increasing quartile from lowest to highest in all groups, with the higher prevalence of elevated levels (≥2 mg/L) in obese subjects. In summary obesity, type 2 diabetes mellitus and metabolic syndrome are multifactorial diseases of considerable heterogeneity and their contribution to CV risk seems to differ substantially.
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HYPOCHOLESTEROLEMIC EFFECTS OF LUPIN PROTEIN AND PEA PROTEIN/ FIBER COMBINATIONS IN MODERATELY HYPERCHOLESTEROLEMIC PATIENTS

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Introductio. The management of individuals with moderate hypercholesterolemia in primary prevention constantly poses the question whether it is more acceptable to treat with drugs or diet

(1; 2). This study was aimed to evaluate the effect of diet supplements, in particular plant proteins (lupin or pea protein), fibers (oat fiber and apple pectin) and their combinations on plasma total and LDL cholesterol levels in low cardiovascular risk patients.
Materials and Methods. A randomized, double-blind, parallel group design was followed: after a 4 week run-in period, patients were randomized into seven treatment groups, each consisting of 25 patients.
Each group consumed two bars containing specific protein/fiber combinations: the reference group consumed casein plus cellulose; in the bars of second and third group lupin or pea proteins replaced casein; in the bars of fourth and fifth group oat fiber or apple pectin replaced cellulose; the sixth and seventh group received bars containing combinations of pea protein and oat fiber or apple pectin respectively. Lipid (Total Cholesterol, LDL-C, HDL-C, Tryglicerides), metabolic (Glucose, Insulin, Adiponectin) and inflammatory (hs-CRP, sICAM-1, IL-6) parameters were determined, during the study.
Results. Bars containing lupin protein + cellulose (-11.6 mg/dL = 4.2%), casein + apple pectin (-15.2 mg/dL = -5.3%), pea protein + oat fiber (-13.5 mg/dL = -4.7%), pea protein + apple pectin (-16.8 mg/dL = -6.4%) resulted in significant reductions of total cholesterol ($p<0.05$), whereas no cholesterol changes were observed in the subjects consuming the bars containing casein + cellulose, casein + oat fiber or pea protein + cellulose. No metabolic and inflammatory parameters changes were noted.
Conclusions. The present study shows for the first time the hypocholesterolemic activity and potential clinical benefits of consuming lupin protein or combinations of pea protein and a soluble fiber, such as oat fiber or apple pectin.
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THE GAIN OF FUNCTION D374Y-PCSK9 MUTANT CAN BE FUNCTIONALLY INHIBITED IN VITRO BY AN EGF-A PEPTIDE

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Introduction. Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to low density lipoprotein receptor (LDLR) and induces its internalization and degradation. PCSK9 binding to LDL-R is mediated through the LDL-R epidermal growth factor-like repeat A (EGF-A) domain. It has been shown that a EGF-A peptide inhibits PCSK9-mediated degradation of LDL-R in HepG2 cells.
Objectives. The aim of this study was to evaluate the effect of a synthetic EGF-A peptide on the LDL-R protein levels in two different hepatic cell lines (HepG2 and HuH7) transiently overexpressing a gain of function mutation of PCSK9.
Methods and Results. A 40mer synthetic peptide of EGF-A domain custom-synthesized was added to the medium of HepG2 and HuH7 cells transiently overexpressing the gain-of-function D374Y PCSK9 mutant which has been associated to an autosomal dominant hypercholesterolemia (ADH3) in humans. D347Y-PCSK9-mediated LDL-R degradation was determined by Western blotting of cell lysates, showing a >85 % LDL-R protein levels reduction. In HepG2 and HuH7 cells the treatment with media containing increasing concentrations of the EGF-A peptide (in the range of 12.5-50 µM) for 18 h restored the LDL-R protein levels.
Conclusions. In summary, these data demonstrate that a synthetic EGF-A peptide is able to efficiently inhibit in vitro the degradation of LDL-R mediated by a gain of function PCSK9 mutant. This data confirm that the inhibition of the extracellular interaction between PCSK9 and the LDLR by using a decoy strategy could represent a valid therapeutic approach for the treatment of Hypercholesterolemia in humans.

HYPOVITAMINOSIS D AND MYALGIA DURING HYPOLIPIDEMIC THERAPY

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Introduction. Side effects may decrease compliance and long term benefits of hypolipidemic therapy. The most common statin side effect (and cause of withdrawal) is myopathy. The role of vitamin D in muscle metabolism is intriguing; in hypovitaminosis D hyperesthesia and myalgias are reversible with supplementation. Aim of our ongoing study is to evaluate the relationship between hypovitaminosis D and myopathy during hypolipidemic treatment and the feasibility of avoiding myalgia with vitamin supplementation.
Materials and Methods. We selected 25 patients (19F and 6M, mean age 62.4, range 40-78) with myalgia during hypolipidemic therapy, low Vitamin D3 levels(<75 nmol/L) and CPK <3XULN, without previous diagnosis of primary myopathy, fibromyalgia, arthritis, PAD, neuropathy, hypothyroidism. At visit 1 subjects underwent a validated questionnaire on muscle clinic and Vitamin D3, Calcium, CPK, PTH determination. Vitamin D3 supplementation was then administered (15000 IU/week for 3 months or 300.000 IU once). At visit 2 after 3 months we repeated clinic assessment, questionnaire and blood analysis.
Results. Before and after supplementation mean values were Vit.D3 34.7±13.7 and 103.2±87.1 nmol/L, PTH 63.3±22.2 and 68.6±23.0 ng/L, CPK 227.9±162.0 and 161±103.6 UI/L, Ca 2.39±0.12 and 2.37±0.13 mmol/L respectively.
According to Vitamin D3 increase muscle clinic improved after supplementation. Questionnnaire showed a decrease of pain score from 11 to 5 with total disappearance of symptoms in more than 30% of subjects, while only one patient didn't show any improvement.
Conclusions. our preliminary results may be clinically relevant due to the poor amount of references in this field. We can hypothesize a direct role of hypovitaminosis D in the appearance of muscle symptoms during hypolipidemic therapy. So, vitamin D3 supplementation might improve muscle symptoms and warrant better compliance to long term treatment in hyperlipidemic patients with vitamin D insufficiency. The prevalence of such patients still needs to be defined.

IMPROVEMENT OF METABOLIC CONDITIONS IN PATIENTS AFFECTED BY TYPE I DIABETES MELLITUS AFTER SHIFT TO SYNERGIC THERAPY WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION AND NUTRITIONAL EDUCATION (CARBOHYDRATES COUNTING): PRESENTATION OF A PRELIMINARY SURVEY

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Aim. Of the study We evaluated some clinical ad anthropometric variables one year after tha beginning of continuous sucutaneous insulin infusion (CSII) and application of CHO counting in patients affected by type I diabetes mellitus.
Materials and Methods. Using the database EuroTouch 9.1, we colleced clinical data coming from 10 patients (5 males, 5 females, average age 38,7 years old, mean diabetes duration 16,5 years). We considered Hba1c, BMI and insulinic requirement (divided into basal insulin and prandial bolus) at baseline and 12 months after the positioning of insulin pump. At the beginning of CSII, patients were given nutitional education with CHO counting by a specialist in Human Nutrition.
Results. One year after the introduction of the CSII and CHO counting, we have noticed a significant reduction of HbA1c (9,75 vs 8,6%, p=0.02), despite we are still far from guidelines target. Its descending trend has been confirmed even if we consider the difference between the value during the year before the therapeutic change and the value found after a year of CSII. BMI remained stable. The CSII brought to a significant reduction of the avarage daily glycemic values and therefore of the HbA1c jusified by an increased theraputic flexibility. The insulinic requirement during 24 hours deminished (39,7 vs 30,3 UI/24 h; p=0.02) with a settlement of basal insulinization and the reduction of the meals injected insulin (23,6 vs 14,2 UI; p=0.004). This has determined an improved sensitivity to insulin action (47.7 vs 61.7 mg/dl/UI) with a better relation CHO/insulin (12.2 vs 15.9 gr CHO/I UI; p=0.02).
Conclusions. the CHO counting allows the patients to identify the most suitable pre-meal insulin bolus, improving HbA1c, sensitivity to insulin action and relation CHO/insulin. The specialist in Human Nutrition finds a preminent place inside the diabetologic team next to the other professional figures.

VITAMIN D AND MORTALITY: THE PROGETTO VENETO ANZIANI (PRO.V.A.) STUDY

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Background. Low vitamin D levels are associated with total and cardiovascular mortality in the American population of NHANES. Aim: To explore the association of low vitamin D levels with total and cardiovascular mortality in a general Italian elderly population.
Research Design and Methods. Vitamin D levels, total and cardiovascular mortality were evaluated in 2829 subjects aged 65 years and older of the Pro.V.A. Study during a mean follow-up time of 4.3±1.3 years.
Results. Of the baseline cohort, 328 subjects (72 out of 1137 men, and 256 out of 1692 women) had vitamin D levels <25 nmol/l, and 1263 subjects (358 men, and 905 women) had vitamin D 25-75 nmol/l. After adjustment for age, sex, season of measurement, parathyroid hormone, calcium, BMI, smoking, physical activity, albumin, LDL and HDL cholesterol, presence of chronic diseases, vitamin D levels <25 and 25-75 nmol/l were associated with increased all cause mortality among all subjects (HR=1.81; 95% CI 1.35-2.43; p=0.000; and HR=1.34; 95% CI 1.09-1.64; p=0.005), among men (HR=1.67; 95% CI 1.05-2.65; p=0.031; and HR=1.31; 95% CI 1.02-1.70; p=0.037), and among women (HR=2.15; 95% CI 1.38-3.35; p=0.001; and HR=1.55; 95% CI 1.07-2.23; p=0.019). After exclusion from the analysis of subjects affected by cardiovascular diseases (n=358), and adjustment for age, sex, season of measurement, parathyroid hormone, calcium, BMI, smoking, physical activity, albumin, LDL and HDL cholesterol, presence of chronic diseases, vitamin D levels <25 and 25-75 nmol/l were associated with increased cardiovascular mortality among all subjects (HR=2.40; 95% CI 1.47-3.94; p=0.000; and HR=1.63; 95% CI 1.14-2.33; p=0.007), among men (HR=1.42; 95% CI 0.59-3.43; p=0.439; and HR=1.49; 95% CI 0.94-2.37; p=0.090), and among women (HR=3.53; 95% CI 1.73-7.18; p=0.001; and HR=1.92; 95% CI 1.04-3.55; p=0.038).
Conclusions. Our findings demonstrate that all cause and cardiovascular mortality are predicted independently by low vitamin D levels in a general Italian elderly population.

MACROPHAGE, BUT NOT SYSTEMIC, APOLIPOPROTEIN E IS NECESSARY FOR MACROPHAGE REVERSE CHOLESTEROL TRANSPORT IN VIVO

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Objective. We previously demonstrated that the complete absence of apolipoprotein E (apoE) in mice leads to the impairment of macrophage reverse cholesterol transport (mRCT) in vivo.

Here we assessed the contribution of macrophage or systemic apoE in this antiatherosclerotic process.
Methods. We discriminated between macrophage and systemic apoE contribution measuring mRCT with a well established assay that traces the cholesterol mobilization from macrophages to plasma, liver and feces. MRCT extent was measured in normal mice injected with apoE null macrophages (apoE/WT), in apoE null mice injected with normal macrophages (WT/apoE) in comparison with normal mice injected with normal macrophages (WT/WT).
Results. In apoE/WT the amount of 3H-cholesterol mobilized along the mRCT pathway was reduced compared to WT/WT mice (%cpm in plasma, liver and feces: 7.49%±0.98 vs 10.61%±1.44; p<0.01). Differently, in WT/apoE mice the process efficiency was fully restored (6.9% ±0.7 vs 4.4%±0.4 in WT/apoE and WT/WT mice respectively). The mechanisms accounting for these results were investigated by evaluating the first step of RCT, cholesterol efflux from cells. Macrophages isolated from apoE null mice showed a defective ability to release cholesterol into the culture medium, whereas the apoB-depleted plasmas from apoE null and normal mice possessed a similar capacity to promote cellular lipid release from cultured macrophages.
Conclusions. Our data demonstrated that expression of apoE in macrophages is sufficient to promote normal RCT even in absence of systemic apoE, and that this role is fully attributable to the promotion of cholesterol efflux from macrophages.

DOES HEPARIN-INDUCED EXTRACORPOREAL LDL PRECIPITATION (H.E.L.P.) APHERESIS IMPROVE DIABETIC FOOT ISCHEMIC ULCERS? A CASE REPORT

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Background. The diabetic foot (DF) is underlied by neuropathy, atherosclerosis of distal arteries, and infection, which result in tissue ulcers and necrosis. Alterations in microcirculatory function and in blood rheology may concur in causing tissue damage. HELP improves plasma viscosity and microcirculatory function by removing a number of proteins, including, but not limited to fibrinogen and lipoproteins. GOAL: To report a pilot case of LDL-apheresis use in the treatment of ischemic gangrene in the DF. CASE: One inpatient Caucasian 65-year-old male subject with bilateral ischemic foot gangrene (left: toe ulcer, Wagner stage IV; right: heel ulcer, Wagner stage IV) and type 2 diabetes mellitus. Personal history was positive for myocardial infarction (3-vessel disease treated with CABG in 2007); peripheral artery disease was documented by angiography. Treatment included bilateral lower limb revascularization by percutaneous angioplasty, antibiotics, strict glucose and metabolic control, supportive medical and nutritional therapy and advanced medications of foot ulcers. Clinical course was one of gradual and steady worsening of both ulcers. A limb salvage attempt was performed with weekly LDL-apheresis by HELP for a total of 12 sessions. Before and after each apheresis session blood lipids, Lp(a), Fibrinogen, C-reactive protein (CRP) were assessed. Transcutaneous oxygen pressure (TcPO2) was measured before, 2, 10 and 12 weeks after initiating LDL-apheresis. RESULTS: Basal laboratory values were: total-cholesterol 113 mg/dl, LDL-C 67 mg/dl, HDL-C 28 mg/dl, Triglycerides 90 mg/dl, Lp(a) 72,7 mg/dl, Fibrinogen 3,59 g/L, CRP 73 mg/L. Basal TcPO2: right foot 19mmHg, left foot 3 mmHg. After each LDL-apheresis serum lipids, fibrinogen and CRP approximately halved. After 12 weeks both foot ulcers were greatly improved (left: Wagner stage 0; right: Wagner stage II). Clinical improvements were mirrored by increased TcPO2 (right: 53 mm Hg; left: 75 mm Hg). Durability of left foot healing and achievement of right foot healing (Wagner stage 0) were documented at 6-month follow-up.
Conclusions. This case report strongly suggests that LDL-apheresis treatment should be investigated as a novel, potential tool to treat ischemic gangrene in the DF.

LOW DENSITY LIPOPROTEIN-APHERESIS FOR TREATMENT OF HYPERCHOLESTEROLEMIC PATIENTS WITH CORONARY ARTERY DISEASE REFRACTORY TO LIPID LOWERING DRUG THERAPY – 15 YEARS EXPERIENCE IN VERONA

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Background. Primary hypercholesterolemia (HC) is a major modifiable risk factor for atherosclerosis and coronary artery disease (CAD). Patients with inheritable HC who cannot be adequately controlled on combination drug therapy are candidates for LDL apheresis. OBIECTIVE: To assess long term LDL-apheresis safety and CAD evolution in HC patients inadequately controlled on drug therapy.
Methods. 10 (7M, 3F) HC consecutive patients (age: 53,5±9,1 years, range 39-66) not responding or intolerant to maximum cholesterol lowering therapy with clinical CAD underwent LDL-apheresis on a regular basis from December 1995 up to august 2010. Before apheresis coronary arteriography documented 3-vessel disease in 4, 2-vessel disease in 2 and single vessel disease in 3 patients, respectively. LDL-apheresis was regularly performed on a fortnight schedule. Two patients have been on treatment for 2 years, 2 patients for 4 years, 2 patients for 6 years, 1 patient for 8 years and 3 patients for >10 years. Before and after each apheresis session blood pressure, heart rate, lipid values and fibrinogen were assessed.
Results. After each apheresis session blood lipids and fibrinogen fell significantly (LDL-Cholesterol -64%, Lp(a) -68%, fibrinogen -63%; p<0.0001 for all). The pre-session LDL cholesterol levels fell significantly by ~19% (p<0.001). PTCA was performed in 2 patients. Patients on treatment for >10 years showed CAD stabilization in subsequent coronary arteriographies. Clinically significant side effects were bleeding in the venipuncture site (2 episodes in 1 patient) and low outlet flow (6 episodes in 4 patients).
Conclusions. LDL-apheresis treatment was safe and well tolerated. Long term LDL-apheresis may stabilize CAD in a number of patients with HC and symptomatic CAD.

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