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ABSTRACT

PHARMACOLOGICAL, ATP-BINDING CASSETTE TRANSPORTER A1 (ABCA1)-DEPENDENT MEMBRANE FREE CHOLESTEROL POOL REDUCTION LEADS TO ATHEROPROTECTIVE MODULATION OF MACROPHAGE FUNCTIONS

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Introduction. Free cholesterol (FC) accumulates in macrophage-derived lipid-laden foam cells contributing to the progression of atherosclerotic plaque. Excess FC is transported to the plasma membrane where it induces a number of deleterious cellular responses including cell death. We have previously demonstrated that this accumulation leads to ruffles formation and impairs macrophage migration in an ABCA1-dependent manner. Probuco, an ABCA1 specific inhibitor, prevented FC deleterious effects. Aim. To investigate whether pharmacological modulation of the ABCA1-dependent membrane FC pool impacts on macrophage proatherogenic functions.

Methods. Cells used were wild type (WT) and ABCA1 knock-out (KO) mouse peritoneal macrophages (MPMs). Pharmacological modulation of ABCA1 was evaluated as cholesterol efflux to apoA-I. Plasma membrane cholesterol was evaluated as oxidase-accessible pool. MCP-1 levels were quantified by standard ELISA assay.

Results. Membrane cholesterol content positively correlates with ABCA1 gene expression in WT, heterozygous and homozygous ABCA1-KO MPMs. We have identified three structural probucon analogues, AGI-1067, compound A and compound B, all three more active than probucon in inhibiting ABCA1 activity (probucon IC₅₀ = 1.80 μM and AGI1067, compound A, B IC₅₀ < 0.5 μM). The two compounds specific inhibitors of ABCA1, compound A and B, inhibited macrophage release of MCP-1 with a higher efficiency than probucon (-32.2%, -33.6% and -22.8%, respectively) and a similar effect was obtained with AGI-1067, an inhibitor of both ABCA1 and ABCG1 (-38.8%). A similar inhibitory effect on ABCA1 activity and on FC-induced MCP-1 release and cytotoxicity in macrophages, was observed also with a non related structural compound berberine.

Conclusions. Our results suggest that specific modulation of ABCA1-dependent FC pool may play a role in regulating macrophage functions involved in atherogenesis.

FATTORI DI RISCHIO LIPIDICI NON CONVENZIONALI PREDITTIVI DELLO SPESSORE MEDIO INTIMALE (IMT) IN UNA COORTE DI PAZIENTI DIABETICI DI TIPO 2

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Lo studio DiAL-ER ha esaminato parametri lipidologici non convenzionali (sottofrazioni lipoproteiche, parametri ossidativi e funzionali) in soggetti sani e affetti da dislipidemie primitive o seconda-

rie, ponendoli in relazione ad altri determinanti di rischio e con un parametro di aterosclerosi subclinica: lo spessore medio-intimale carotideo (cIMT).

Scopo della ricerca: è stato valutare nei soggetti con dislipidemia diabetica la relazione tra i FR convenzionali, le sottofrazioni lipoproteiche (LDL e HDL, tramite separazione all'ultracentrifuga ed elettroforesi PAGE) e l'ossidabilità delle LDL vs. cIMT, marker di danno precoce aterosclerotico.

Popolazione e metodi: sono stati reclutati 148 pazienti (86 M e 62 F) di età media pari a 65±10 anni affetti da diabete tipo 2 e dislipidemia. Si sono considerati parametri antropometrici, emodinamici, metabolici, sottofrazioni di HDL e LDL, concentrazione di lipoproteine ossidate circolanti (LDLox) e cinetica di ossidazione delle LDL. Il cIMT è stato misurato con tecnica ecocolor Doppler. L'analisi è stata condotta nell'intera coorte e dopo suddivisione per sesso con l'impiego di un modello di regressione lineare multipla (p=0.05).

Risultati. Nella coorte M e F presentavano cIMT non significativamente diverso (1,05±0.28 mm vs. 0.96±0.20 mm). Nell'ambito di vari modelli testati, l'analisi multivariata ha evidenziato tra i fattori predittivi di IMT (R²=0.37): età, sesso maschile, LDLox, presenza di large HDL e c-HDL. Nella popolazione femminile (R²=0.44) si confermava il ruolo di età, LDLox e large HDL mentre in quella maschile, in cui il modello appariva complessivamente dotato di scarsa predittività (R²=0.29) i parametri lipoproteici ed ossidativi non risultavano significativi rimanendo significativa solo l'età.

Conclusioni. Lo studio evidenzia un ruolo importante delle sottofrazioni lipidiche e dei parametri di ossidazione lipidica nella predizione della malattia cardiovascolare precoce con un impatto diverso nei due sessi.

IS THE ENLARGEMENT OF BRACHIAL ARTERY DIAMETER A NOVEL MARKER OF ATHEROSCLEROTIC RISK?

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Background. During the atherogenic process, the arterial diameter measured in plaques free areas tends to enlarge. This enlargement does not reflect the process defined "vascular remodeling", which occurs primarily as a local response to the rheological changes induced by the presence of atherosclerotic plaques, but rather it occurs as simple compensatory response of arteries to the presence of atherosclerosis risk factors. Several studies suggested this arterial enlargement as a further surrogate marker of atherosclerosis. In a recent study (1), we have shown that the addition of the inter-adventitia common carotid artery diameter (ICCAD) measured in plaque-free areas to algorithms for the assessment of global cardiovascular risk improves the patient's risk stratification. However, carotid arteries are rarely free of atherosclerotic lesions, especially in adult or elderly subjects, and even if the measures are taken in plaque free areas, it can not be excluded the presence of plaques in the surroundings which might alter the vessel rheology, thus being the indirect responsible of the enlargement observed. Some studies have recently evaluated the diameter of other arterial districts known to be less prone to the development of atherosclerosis lesions. Most of these studies, focused on the brachial artery diameter (BAD), indicate that the arterial enlargement is a

generalized phenomenon, and suggest that, as carotid diameter, also the enlargement of this arterial district may be useful to further improve the prediction of vascular events. All these studies, however, have been carried out in relatively small samples. In addition, limited information is available regarding the determinants of the enlargements evaluated simultaneously in different vascular districts.

Aim of the Study. To validate, in a large sample, the role of BAD as an independent marker of atherosclerosis and to investigate whether the addition of BAD measurements to ICCAD measurements may actually offer additional information for the definition of patients' cardiovascular risk profile.

Methods. 4641 patients (44.6% women and 55.4% men; age (mean \pm SD) 58 \pm 13 and 55 \pm 13, respectively) have their BAD, ICCAD and carotid Intima media thickness (C-IMT) measured by B-Mode ultrasound. Measurements have been taken during the first visit at the Centro Dislipidemie E. Grossi Paoletti, (Ospedale Ca' Granda di Niguarda) or at the Centro Cardiologico Monzino, IRCCS. Both BAD and ICCAD were measured in plaque free areas. A total of 4271 subjects were asymptomatic, whereas 335 (64 women and 271 men) experienced a myocardial infarction and 35 (11 women and 24 men) a stroke.

Results. BAD was associated with the prevalence of vascular events in both women and men. After adjustment for age, traditional risk factors, C-IMT and ICCAD, this associations persisted in women (O.R and CI: 2.2 [1.1-4.4]; $p < 0.05$) but not in men (O.R and CI: 1.1 [0.8-1.7]; $p = ns$). When the analysis was performed considering myocardial infarction and stroke separately, it becomes clear that the observed significant association was mainly due to association with myocardial infarction (O.R and CI: 2.6 [1.2-5.6]; $p < 0.05$). BAD was closely associated with ICCAD (Beta of about 0.30 \pm 0.03; $P < 0.0001$, in both sexes). Despite this, determinants of the enlargement of the two vascular districts were very different. For example, the relationship between BAD and the Framingham risk score was two times lower than that observed with ICCAD).

Conclusions. The BAD is an independent marker of myocardial infarction, which, at least in women, may provide information which is complementary to that coming from vascular risk factors and ICCAD.

Reference

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EARLIER VASCULAR DAMAGE IS ASSOCIATED WITH A MORE BENEFICIAL IMPROVEMENT OF PRO-ATHEROGENIC PROFILE AFTER SMOKE CESSATION

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The aim of this work was to evaluate whether smoke cessation may result in changes in monocyte biglycan (BGN)-mRNA expression in young smokers without additional CAD risk factors, with or without increased carotid intima-media thickness (cIMT). Monocyte expression of BGN, a multivalent proteoglycan providing structure and signals, is enhanced in subjects with CAD risk factors. Seventy-five cigarette smokers (mean age 24.9 \pm 3.6 years) and 60 matched controls were enrolled. Smokers were divided into 3 groups stratified for cIMT values (G1: ≤ 0.99 mm; G2: ≥ 1 mm < 1.3 , G3 ≥ 1.3 mm, respectively). Plasma concentrations of interleukin-6 (IL-6), C-reactive protein (hs-CRP), fibrinogen and lipids, blood pressure (BP) and BGN-mRNA in circulating monocytes were

measured at baseline (T0) and after 9-months smoke cessation (T1). To evaluate the influence of smoking on study parameters a score of smoke exposure was estimated (SEIx). Arterial stiffness (AS) values (AIx and PWV) were also measured. At baseline, fibrinogen, CRP, BGN-mRNA values were higher in smokers than controls, while HDL-C was lower; this difference was enhanced in G2 and particularly in G3. Diastolic BP (DBP) was increased in G2 and G3 and IL-6 in G3.

At T1, in each of three groups, fibrinogen, CRP and IL-6 were reduced with respect to baseline, and HDL-C was increased. DBP was reduced in G2 and G3; BGN-mRNA were decreased in G1 and G2, while it did remain unchanged in G3. AIx and PWV were reduced in G1 and G2 with respect to baseline, whereas in G3 they remained unchanged. The regression analysis suggested that at T0 the main predictors for BGN-mRNA were inflammation and SEIx, and that at T1 BGN-mRNA was associated with PWV, HDL-C and DBP. These findings suggest that smoke cessation may reduce the expression of monocyte BGN-mRNA and improve pro-atherogenic profile, particularly in subjects presenting with an earlier vascular involvement.

STUDIO DELL'EFFETTO ANTIPROLIFERATIVO DEI DONATORI DI OSSIDO D'AZOTO FUROSSANI IN CELLULE MUSCOLARI LISCE. POSSIBILI FARMACOFORI PER NUOVE MOLECOLE ANTIATEROSCLEROTICHE?

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L'aterosclerosi è una patologia in cui ossidazione, alterata produzione di NO e proliferazione delle cellule muscolari lisce (CML) svolgono un ruolo essenziale. Pertanto abbiamo sintetizzato molecole NO-donatrici (furossani) in grado di rilasciare il tessuto aortico in modo NO-dipendente e di inibire la proliferazione di CML. Per comprendere le basi molecolari di questo effetto, dopo aver bloccato con un fenile la posizione 4 dell'anello furossanico, abbiamo dimostrato come l'inibizione della proliferazione correli con le proprietà elettronattrici del gruppo in posizione 3 (R). Esperimenti condotti sui 3-fenil-4-R-furossani corrispondenti e sui des-NO analoghi furazanici hanno dimostrato come la potenza inibitoria dei primi sia molto inferiore ed addirittura nulla negli ultimi, rispetto ai 4-fenil-3-R-furossani. Poiché evidenze sperimentali escludono che la loro attività inibitoria sulle CML sia mediata da NO attraverso la via della guanilatociclastasi o quella delle poliamine, abbiamo effettuato esperimenti di proliferazione in cui i furossani vengono cosomministrati alle CML con una sospensione di globuli rossi (25 ul/ml medium), quale sequestrante di NO. Questo trattamento previene completamente l'inibizione della proliferazione da parte dei furossani, dello SNAP (composto NO-donatore classico), ma non quella ottenuta con bifosfonati e statine, il cui effetto antiproliferativo non è NO-mediato. Stiamo pertanto identificando, mediante analisi proteomica, proteine cellulari S-nitrosilate bersaglio dei furossani, implicate nella progressione del ciclo cellulare (Williams et al., 2009: modificazione da parte dei furossani della tioredoxina-glutatione-riduttasi negli Schistosomi). I presenti risultati, la modulabilità del rilascio di NO e la comprensione del meccanismo d'azione dei furossani potrà permettere di sfruttarli nell'ibridazione con antiossidanti o farmaci in grado di controllare

altri aspetti del processo aterosclerotico, per sviluppare nuove terapie. I risultati preliminari finora ottenuti con ibridi furossanici/antiossidanti evidenziano come nelle nostre condizioni sperimentali queste molecole, seppure con diversa potenza, inibiscano la crescita delle CML, al contrario delle porzioni antiossidanti isolate (fenoli, vitamine E/C, carnosina, edaravone, melatonina), suggerendone la loro validazione in modelli animali.

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CORRELAZIONE TRA ATTIVITÀ DELL'ENZIMA PARAOXONASI E LIVELLI DELLA PROTEINA C REATTIVA NELL'OBESITÀ

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Elevati livelli di lipoproteine ad alta densità (HDL) sono associati ad un ridotto rischio di patologie cardiovascolari. Una attenzione crescente è rivolta alla qualità e funzionalità delle HDL, piuttosto che ai livelli plasmatici di HDL-colesterolo. Infatti le HDL promuovono l'efflusso di colesterolo cellulare dalle cellule periferiche, svolgono un'azione antitrombotica, antiossidante e antiinfiammatoria. Alterazioni della composizione lipidica e apoproteica associate a numerose condizioni patologiche (infiammazione sistemica, stress ossidativo) alterano le funzioni protettive esercitate dalle HDL e le convertono in lipoproteine pro-infiammatorie e pro-aterogene.

L'enzima paraoxonasi-1 (PON1) associato alla superficie delle HDL protegge le lipoproteine e le membrane dall'ossidazione e riduce la risposta infiammatoria e pro-aterogena indotta dai prodotti di perossidazione lipidica. Studi precedenti hanno dimostrato che una bassa attività della PON1 è associata ad elevati livelli plasmatici della proteina C-reattiva (CRP), una proteina sintetizzata in risposta all'infiammazione.

Al fine di investigare la relazione tra l'attività di PON1 e i livelli di CRP, abbiamo valutato il rapporto PON1/CRP in 25 soggetti normopeso e 22 soggetti obesi (BMI compreso tra 30 e 71 kg/m²). I risultati hanno dimostrato che il rapporto PON1/CRP è significativamente più basso nei soggetti obesi rispetto ai controlli (158±26 vs 942±106, rispettivamente, p <0,001). Il rapporto PON1/CRP più basso (56±11) è stato osservato in soggetti con BMI > 50 (n=7). Nei soggetti con BMI compreso tra 30-39 (n=6) il rapporto era 290±56, valori intermedi (PON1/CRP=150± 59) sono stati osservati nei soggetti obesi con BMI compreso tra 40<BMI>49 (n=9).

Una diminuzione del rapporto PON1/CRP è stato osservato in soggetti diabetici e in pazienti con patologie renali. I nostri risultati confermano la relazione tra l'attività di PON1 e i livelli di CRP e suggeriscono che il rapporto PON1/CRP potrebbe rappresentare un utile indicatore dell'alterato equilibrio tra i processi infiammatori e le proprietà antiossidanti delle HDL.

THE EFFECTS OF TOBACCO SMOKE AND OF THE SOCIAL CLASS ON CAROTID INTIMA MEDIA THICKNESS (C-IMT) AND ON C-IMT PROGRESSION ARE STRONGER IN WOMEN THAN IN MEN

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Background. The harmful effect of smoking on atherosclerosis and cardiovascular health is well established. Educational campaigns have been successful in reducing the number of smokers in men but not in women, where the number of smokers (initially lower than men) is even increasing.

Aim of the Study. To investigate the gender differences in the association of tobacco smoke with subclinical atherosclerosis and atherosclerosis progression also taking into account the effects of other variables strongly associated with tobacco smoke: C reactive protein (CRP) and number of white blood cells (WBC) as inflammation markers, and education as an index of social class.

Methods. The IMPROVE Study cohort includes 1694 men and 1893 women (age 54-79 yr) at high risk of cardiovascular disease of five European countries. Baseline mean and maximum IMT of the left and right common carotids, bifurcations and internal carotid arteries and the fastest IMT-progression (15 months of follow up) detected in the whole carotid tree regardless of its location were computed. Associations were assessed by multivariable analysis adjusting for conventional cardiovascular risk factors and recruiting centre.

Results. Pack-years, a lifelong index of tobacco exposure, significantly associated with baseline C-IMT in both genders. However, the estimated C-IMT increase for each pack-year was more than double in women than in men (3.7±0.7 vs. 1.5±0.5 µm) with a significant gender × dose interaction (P=0.01). Moreover, the estimated increase in the fastest C-IMT progression associated with a unit of cigarettes/day, an index of daily dose of tobacco exposure, was more than five-fold in women than in men (5.5±1.3 vs. 1.0±1.3 µm/yr), (P-int =0.008). Also the relationships between C-IMT and CRP (P-int =0.015), WBC (P-int =0.011) and education (P-int =0.014) were different in men and women. Gender differences were also observed considering the relationships between current smoking and CRP (P-int =0.045) and WBC (P-int =0.049). Finally, a significant gender difference was also found in the relationship between education and smoking exposure (P-int =0.0003).

Conclusions. The effects of tobacco smoking on cross-sectional subclinical atherosclerotic burden, and on carotid atherosclerosis progression appear to be more harmful in women than in men, prompting studies on gender specific mechanisms and development of preventive actions expressly oriented to women. Inflammation and social class seems to be implicated in the complex interrelation between tobacco smoke, gender and subclinical atherosclerosis.

SIX YEARS TELOMERE SHORTENING IS ASSOCIATED WITH INCREASED INCIDENCE OF EXTRA-CARDIAC DAMAGE AND CARDIOVASCULAR PROGNOSIS

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Leukocytes Telomere Length (LTL) is an important determinant of telomere function and cellular replicative capacity. Recent findings support an association of LTL with age-related diseases, including coronary heart disease (CHD). The association between prospective telomere shortening (TS) and the progression of common carotid intima-media thickness (CCA-IMT), a marker of atherosclerosis, is unknown.

To this aim, we measured mean LTL in 768 subjects (462 female and 306 male) at enrollment and after 6-years follow-up. CCA-IMT was determined and the extra-cardiac damage was defined in the presence of CCA-IMT increase above 0.9 mm. Those with incident cardiovascular events (CVE) were noted. Genotype in TERT A>C (Telomerase Reverse Transcriptase) and ACYP2 C>A (Acylphosphatase 2), GWAS hits for LTL, were determined.

Mean baseline LTL was 1.25±0.92 bp (median, 1.14) and, after follow-up, was 0.70±0.37 bp (median, 0.70). Median TS was 0.078 bp per year and 0.46 bp during follow-up. Therapies did not affect TS. After adjustment for classical risk factors, TS was associated with incident extra-cardiac vascular damages (beta =1.48; p=0.05/year shortening or beta =0.25 p=0.05 for overall shortening) but not with incident CVE. Individuals homozygous for the TERT rs2736100 minor allele had significant TS (beta =0.111, p=0.003) while carriers of ACYP2 rs11125529 minor allele were protected against incident CVE (HR=0.506 [0.272-0.941, 95% C.I.], p=0.031). In summary, we show for the first time that TS is associated with the incidence of extra-cardiac damage in a large free-living population. Furthermore, genetic analysis suggest that the relationship between telomere shortening and CVD may be causal.

PNPLA3 GENE VARIANTS AND CARDIOMETABOLIC PHENOTYPES IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims. Many studies reported a strong relationship between non alcoholic fatty liver disease (NAFLD) and metabolic syndrome. Moreover, nonsynonymous variant I148M located in PNPLA3 (Patatin-like phospholipase domain-containing protein 3, also known as Adiponeutrin) gene has been reported to be associated with NAFLD and with a more severe liver damage. Our

purpose was to assess the influence of PNPLA3 variant on cardio-metabolic phenotype in a large series of patients with NAFLD.

Methods. NAFLD was diagnosed by ultrasonographic Hamaguchi's criteria, in subject with no history of alcohol abuse. 211 subjects were enrolled and genotyped for PNPLA3I148M variant determined by TaqMan assays. Metabolic syndrome was defined according to the ATP III modified criteria.

Results. PNPLA3 genotype frequencies were II=45.3%, IM=40.5%, MM=14.2% and the overall prevalence of MS was 68%. MS was more frequently observed in PNPLA3 wild type allele carriers (II=71.6% vs IM=72.1% vs MM=50% [p=0.024]). Odds ratio for MS was 3.3 times lower in MM carriers as compared to IM and II alleles carriers. Median waist circumference (cm) and serum triglycerides (mg/dl) were higher in I allele carriers [110 (103.5/118) vs 105 (101/113.5) vs 106 (96.7/118.5), p=0.065 and 157 (112/193) vs 141 (107.7/185) vs 111.5 (90.7/148.7), p=0.006, respectively], while mean ALT (UI) was higher in M carriers (26 (19/35) vs 30.5 (22/45) vs 30 (22.7/40.5) [p=0.014]). MM carriers had lower median HOMA-IR and higher median HDL-C (mg/dl) compared to wild-type, although not at statistically significant level (2.8 vs 3.6 and 50.5 vs 45, respectively). Framingham cardiovascular risk score was significantly higher in II vs MM carriers (9% vs 4% p=0.024 respectively). Body mass index, blood pressure and other biochemical parameters did not differ across genotypes.

Conclusions. Subjects with NAFLD carrying PNPLA3 M variant are at risk to develop more severe liver disease but they show a lower prevalence of metabolic syndrome and reduced cardiometabolic risk.

IPERTENSIONE IN GRAVIDANZA: OUTCOMES EMODINAMICI IN UN AMPIO CAMPIONE DI PAZIENTI AMBULATORIALI

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Background. L'ipertensione in gravidanza è una importante causa di mortalità materna e fetale e costituisce un fattore di rischio per lo sviluppo di malattie cardiovascolari a distanza. Le linee guida suggeriscono di trattare farmacologicamente l'ipertensione severa, meno evidenze si hanno invece sull'ipertensione lieve-moderata.

Obiettivo. Determinare i principali fattori prognostici indipendenti degli outcomes materno e fetali in un ampio campione di pazienti ambulatoriali affette da ipertensione lieve-moderata rispetto a gravide non ipertese.

Metodi. Delle 906 pazienti incluse abbiamo raccolto età, BMI, terapia farmacologica (incluso ASA), settimana del parto, numero di gravidanza, parità, livelli di pressione arteriosa ad ogni trimestre e al momento del parto, complicanze materne (sviluppo di preeclampsia, ricovero in terapia intensiva, morte) e fetali (sindrome da distress respiratorio, restrizione di crescita intrauterina, ricovero in terapia intensiva neonatale, aborto). Le pazienti sono state divise in base al tipo di disturbo ipertensivo presentato in normotese (327), preeclamptiche (213), pazienti con ipertensione cronica (221) e pazienti con ipertensione gestazionale (145).

Risultati. Le complicanze materne sono state riscontrate nel 24,8% dei casi, mentre per la prole gli outcomes avversi sono stati registrati nel 10,4% dei casi. All'analisi di regressione logistica, i determinanti predittivi indipendenti dell'outcome materno individuati sono PAD (OR=1,06; CI95%=1,03-1,05), parità (OR=0,222; CI95%=0,08-0,63), terapia farmacologica con α -metildopa (protettiva vs. al-

tri farmaci: OR=0,09; CI95%=0,02-0,35), numero di gravidanze (OR=6,43; CI95%=1,69- 24,5) e BMI (OR=2,831; CI95%=1,31- 6,13). Per quanto riguarda l'outcome fetale, i predittori alla regressione logistica risultavano essere terapia farmacologica (α -metildopa OR=0,179; CI95%=0,04-0,81 e Nifedipina OR=0,105; CI95%=0,018-0,612), PAD al parto (OR=1,033; CI95%=1,005-1,062) e numero di gravidanze (OR=1,759; CI 95%=0,446-6,930).

Conclusioni. La terapia farmacologica è risultata efficace nel ridurre le complicanze materne e fetali e si è visto che l'aumento della PAD al parto è correlato con un forte aumento del rischio di sviluppare complicanze materno- fetali.

TERAPIA "ORFANA" CON NIACINA/ LAROPIPRANT IN PAZIENTI AFFETTI DA IPO-ALFA LIPOPROTEINEMIA O IPERCOLESTEROLEMIA FAMILIARE GIÀ COMPLICATE DA CARDIOPATIA ISCHEMICA

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Introduzione. Ad oggi i livelli di colesterolo HDL sono considerati un importante fattore di rischio indipendente di malattia coronarica. L'associazione tra acido nicotinico a lento rilascio e Laropiprant (ENR/LRPN), fino alla data del suo ritiro dal commercio, ha rappresentato un possibile approccio terapeutico. Abbiamo valutato sostenibilità e efficacia di ENR/LRPN in pazienti affetti da dislipidemia già complicata da cardiopatia ischemica precoce.

Materiali e Metodi. Durante il breve periodo di disponibilità dell'ENR/LRPN sono stati seguiti 32 soggetti (età media 61±7; 74% maschi) con dislipidemia e coronaropatia cronica, già in trattamento con la massima terapia ipolipemizzante tollerata. Nelle prime 4 settimane è stata somministrata una dose di 1 gr/giorno di ENR/LRPN, aumentata a 2 gr/giorno per il restante periodo (12 settimane). Sono stati valutati: variazioni di profilo lipidico, funzione epato-renale e insorgenza di eventi avversi. In specifici sottogruppi (9 soggetti affetti da ipercolesterolemia familiare e 5 soggetti affetti da ipo-alfa lipoproteinemia) tolleranti alla terapia con ENR/LRPN è stata analizzata l'efficacia del trattamento.

Risultati. Durante il periodo di osservazione 16 pazienti hanno interrotto l'assunzione di ENR/LRPN per l'insorgenza di effetti collaterali (5 pazienti: cefalea, astenia e disturbi gastro-enterici; 3 pazienti: miopia con incremento dei CPK; 2 pazienti: orticaria; 2 pazienti: sviluppo di diabete mellito; 2 pazienti: vertigini; 1 paziente: epatite acuta; 1 paziente: palpazioni), 2 pazienti hanno sospeso la terapia senza una causa apparente.

Trascorse 16 settimane di trattamento, nei pazienti affetti da ipercolesterolemia familiare, si osservano variazioni significative nelle concentrazioni plasmatiche di Trigliceridi (-31.2% p<0.05), HDL (+16% p<0.05) ed Lp(a) (-25% p<0.05). Nel sottogruppo con ipo-alfa lipoproteinemia si osserva, invece, il solo aumento significativo dei livelli delle HDL (+27% p<0.05).

Conclusioni. Pur trattandosi di un campione estremamente ridotto, i risultati mostrano come, a fronte di una frequenza importante di effetti collaterali, l'associazione ENR/LRPN potesse rappresentare un'efficace opzione terapeutica per i pazienti dislipidemici.

SIMVASTATIN MODULATES AORTIC VASCULOPATHY IN AN ANIMAL MODEL OF SYSTEMIC SCLEROSIS

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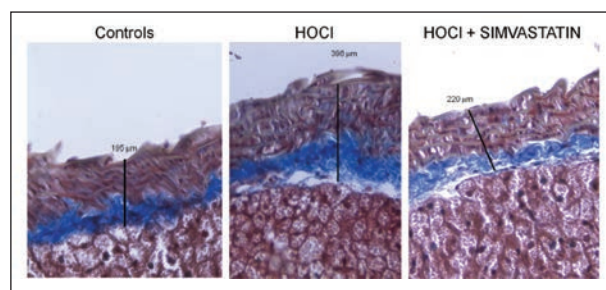
Background. Systemic sclerosis (SSc) is a multisystem autoimmune disease characterized by vasculopathy and organ fibrosis. Although many previous studies highlighted microvascular alterations in SSc, a growing body of evidence exists for structural and functional abnormalities in the macrovascular circulation. Recent reports shows that in SSc patients macrovasculopathy occurs preferentially at the forearm and aorta.

Aim of the study was therefore to evaluate the effect of simvastatin administration on aortic intima-media (IM) thickness and ratio in a murine model of systemic sclerosis.

Methods. SSc-like illness was induced in BALB/c mice by daily subcutaneous injections of HOCl as an oxidant stress for 6 weeks. Mice (n=24) were randomized in three arms to treatment with either HOCl (n=10); HOCl plus simvastatin (n=9); or vehicle alone (n=5). Simvastatin treatment was initiated 30 minutes after HOCl subcutaneous injection (40 mg/kg) continuing daily for the 6 weeks. Thoracic aorta was evaluated by histological methods. IM thickness and ratio were measured for statistical analysis.

Results. In HOCl treated mice aortic IM thickness was significantly higher than controls, showing an increase of 104% (p<0.0001). Treatment with simvastatin diminished this increase by 92% (p<0.0001). Simvastatin treated animals had a significantly thinner intima layer (-9%, p<0.0001) and media layer (-197%, p<0.0001) compared to HOCl group. IM ratio was also decreased in HOCl treated mice compared to controls (0.75 vs 1.74, p<0.0001) and significantly increased by simvastatin administration (1.61 vs 0.75, p<0.0001).

Conclusion. Administration of simvastatin moderates the increase of IM thickness in this animal model of SSc. Further analysis on IM ratio suggests that aortic media layer is thickened in HOCl treated animals and this increase can be prevented by simvastatin.



PENTRAXIN 3 DEFICIENCY IS ASSOCIATED WITH INCREASED ARTERIAL THROMBOSIS IN ANIMAL MODELS

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PTX3 is a biomarker of cardiovascular diseases and exerts protective functions in acute myocardial infarction and atherosclerosis. Studies in animal models have recently identified several proteins targeted by PTX3 that are potentially involved also in arterial thrombosis thus prompting us to investigate its involvement in this event. PTX3 KO mice showed a 60% reduction in carotid artery blood flow with a greater thrombus formation compared to 20% of WT mice ($p < 0,01$) following arterial thrombosis induction. This effect was independent of an altered hemostatic environment or of an impaired platelet activation. As PTX3 modulates P-selectin activation during lung inflammation we investigated whether this interaction is involved in arterial thrombosis. P-selectin KO/PTX3 KO mice showed a significant reduction in carotid artery blood flow and increased arterial thrombus formation (similar to that of PTX3 KO mice) compared to P-selectin KO animals ($p < 0,01$). This finding suggests a PTX3 effect independent on P-selectin modulation. To clarify the contribution of PTX3 produced in the vascular wall in arterial thrombosis, bone marrow transplantation (BMT) experiments were performed. PTX3 KO animals with PTX3 KO or WT bone marrow (PTX3 KO/BMT PTX3 KO or PTX3 KO/BMT WT) and their controls (WT/BMT PTX3KO or WT/BMT WT) were generated. WT/BMT PTX3 KO behaved similarly in terms of arterial thrombosis to WT/BMT WT (50% reduction in carotid blood flow, $p = n.s.$) In contrast, PTX3 KO/BMT WT showed a 70% reduction compared to WT/BMT WT ($p < 0,01$) and a similar carotid occlusion to PTX3 KO/BMT PTX3 KO.

Indeed PTX3 was shown to bind collagen and to dampen its ability to promote platelet adhesion and aggregation by about 40% ($p > 0,001$), an effect related mainly to the C-terminal domain. Finally, exogenous administration of human recombinant PTX3 reverted the pro-thrombotic phenotype in PTX3 KO mice and was shown to dampen arterial thrombosis also in wild type animals.

In conclusion, PTX3 deficiency is associated with increased arterial thrombosis via modulation of vascular thrombogenicity.

ASSOCIAZIONE TRA CONCENTRAZIONI PLASMATICHE DI ACIDO URICO E MORTALITÀ CARDIOVASCOLARE IN PAZIENTI ANZIANI

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Introduzione. Il ruolo dell'acido urico nell'uomo appare controverso. Nonostante abbia attività antiossidante, diversi studi hanno

mostrato una sua relazione con disfunzione endoteliale, marcatori di infiammazione sistemica, malattie cardio-cerebrovascolari e mortalità. Lo scopo di questo studio è stato valutare l'associazione tra concentrazioni di acido urico e mortalità totale e cardiovascolare in un campione di soggetti anziani estratti dalla popolazione.

Materiali e Metodi. Abbiamo esaminato l'associazione tra livelli plasmatici di acido urico e diverse caratteristiche generali, patologiche ed ematochimiche di 1.043 soggetti di età ≥ 65 anni arruolati nello studio HDL. Attraverso un'analisi di regressione di Cox aggiustata per potenziali fattori di confondimento, abbiamo riscontrato che i pazienti con più elevate concentrazioni di acido urico presentano maggiore mortalità cardiovascolare (rispetto al primo quartile 1.8-4.2 mg/dl; terzo quartile 5.1-5.9 mg/dl HR 2.0, IC 95% 1.13-3.54; quarto quartile 6-15 mg/dl HR 1.85, IC 95% 1.02-3.35). I livelli di acido urico non sono invece apparsi predittori significativi di mortalità totale.

Risultati. Maggiori concentrazioni di acido urico sono risultate essere significativamente e positivamente correlate con sesso maschile, età avanzata, circonferenza vita, body mass index, ipertensione, indici di flogosi (globuli bianchi, PCR, IL-6 e IL-18), clearance della creatinina e trigliceridi e inversamente correlate con emoglobina e colesterolo HDL. Attraverso un'analisi di regressione di Cox aggiustata per potenziali fattori di confondimento, abbiamo riscontrato che i pazienti con più elevate concentrazioni di acido urico presentano maggiore mortalità cardiovascolare (rispetto al primo quartile 1.8-4.2 mg/dl; terzo quartile 5.1-5.9 mg/dl HR 2.0, IC 95% 1.13-3.54; quarto quartile 6-15 mg/dl HR 1.85, IC 95% 1.02-3.35). I livelli di acido urico non sono invece apparsi predittori significativi di mortalità totale.

Conclusioni. In soggetti anziani concentrazioni di acido urico > 5 mg/dl risultano essere associate ad un maggiore rischio di morte per cause cardiovascolari a 9 anni indipendentemente dalla presenza dei classici fattori di rischio cardiovascolari.

I LIVELLI DI PTH CORRELANO CON IL DANNO ATEROSCLEROTICO NELLA MALATTIA RENALE CRONICA IN FASE PRE-DIALITICA? NOSTRA ESPERIENZA

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Introduzione. In Letteratura è riportato che l'iperlipidemia, oltre ad avviare un'azione di "bioattività pro-infiammatoria" che innesci la patogenesi dell'aterosclerosi, possa indurre la perdita di tessuto osseo inibendo il recettore per l'ormone paratiroideo (PTH1R) ed interferendo con la differenziazione degli osteoblasti. È stato dimostrato che gli effetti anabolizzanti ossei del PTH sono ridotti nei topi con iperlipidemia e gli stessi effetti possono essere ripristinati dalla somministrazione di "antiossidanti". Oggigiorno da più parti vengono proposti lavori scientifici che accostano il "danno cardiovascolare aterosclerotico" al disordine del metabolismo minerale osseo, soprattutto nei soggetti con malattia renale cronica, focalizzando sempre più l'attenzione sulla cosiddetta CKD-MDB (Chronic Kidney Disease - Mineral Bone Disorders). Tuttavia, la CKD-MDB non risulta ancora correlata ad un eventuale ruolo attivo su di essa da parte di una dislipidemia. Abbiamo pensato di condurre uno studio osservazionale su una coorte di soggetti con CKD, alla ricerca di eventuali correlazioni tra Iperparatiroidismo, Dislipidemia e danno CV aterosclerotico.

Materiali e Metodi. Abbiamo valutato la situazione clinico-metabolica di 138 soggetti (78 M e 60 F) con età anagrafica di 73 aa., in stato di insufficienza renale cronica avanza (Stadio IIIb-IV), in fase "pre-dialitica". I parametri presi in esame sono stati:

- 1) presenza/assenza di dislipidemia;
- 2) presenza/assenza di danno CV aterosclerotico accertato (pre-

gresso IMA o evidenze strumentali di cardiopatia ischemica, pregresso stroke o evidenze strumentali di aterosclerosi carotidea, pregressi interventi chirurgici di rivascularizzazione o evidenze strumentali di ipoperfusione degli arti);

3) valori sierici di iPTH.

Risultati. Abbiamo riscontrato che i soggetti con dislipidemia, oltre a presentare una più elevata prevalenza di danno cardiovascolare aterosclerotico, presentavano più elevati valori di iPTH. Riteniamo che sia opportuno condurre degli studi controllati al fine di apportare maggiore chiarezza nel campo specifico.

ARTERIOPATIA UREMICA CALCIFICA E T.A.O. IN DIALISI: NOSTRE ESPERIENZE

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Introduzione. La Calciphic Uremic Arteriopathy (CUA) è una rara sindrome caratterizzata da deposizione di calcio nelle pareti dei vasi di piccole e medie dimensioni del derma e del tessuto sottocutaneo. La malattia, conosciuta anche come "calciphylaxis", colpisce soprattutto i pazienti con malattia renale allo stadio terminale ed è caratterizzata dalla presenza di ulcerazioni cutanee dolorose. Essa molto spesso sfugge ad una diagnosi precoce ed è correlata con un elevato tasso di mortalità. La patogenesi della CUA non è ancora ben definita, anche se sono stati segnalati diversi fattori di rischio, tra cui la terapia anticoagulante con dicumarolici, soprattutto se utilizzata nei soggetti con alterazioni del metabolismo calcio-fosforo.

Materiali e Metodi. Abbiamo rivalutato la casistica, osservata in un arco temporale di 25 aa. di un Centro Dialisi che assiste iterativamente circa 50 emodializzati, alla ricerca dei casi di CUA.

Risultati. Abbiamo trovato 4 casi, verificatisi tutti in soggetti in TAO. In tutti i casi la CUA era localizzata alle gambe. L'approccio terapeutico è risultato variegato per ogni caso, contemplando oltre la terapia farmacologica emoreologica più vantaggiosa e la correzione del metabolismo minerale, anche l'impiego dell'ossigenoterapia iperbarica. Il primo caso, riguardante una giovane donna sottoposta ad impianto di protesi valvolare aortica, ha avuto una devastante evoluzione verso l'exitus. Gli altri tre casi riguardavano pazienti in trattamento in TAO per Fibrillazione Atriale. Nel primo caso vi è stata l'impossibilità di sospendere la TAO in relazione alla patologia cardiaca, mentre negli altri casi si è scelto di sostituire la TAO con altra terapia, osservando in tutti i casi la lenta regressione delle ulcerazioni cutanee. In base alle nostre osservazioni, riteniamo che:

- 1) la prevalenza della CUA sia considerevole ed essa sia meritevole di "early attention";
- 2) l'utilizzo della TAO in dialisi debba essere molto oculato.

OXIDATIVE STRESS IN DYSLIPIDEMIC CHILDREN

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Introduzione. Oxidative stress is implicated in the pathogenesis of endothelial dysfunction and atherosclerosis progression. This event cascade may start in childhood and is correlated with hypercholesterolemia. Data on Oxidized LDL (Ox-LDL), a marker of oxidative stress, are poorly checked in children besides controver-

sial. Aim of the study was to evaluate the Ox-LDL status in children affected by primary hypercholesterolemia.

Methods. 44 hypercholesterolemic children (10.4±2.8 years, BMI 21±1.9) and 20 normocholesterolemic children (sex and age matched) afferring to our department, were included in the study. On the basis of international criteria they were affected by Familial Hypercholesterolemia (FH) (n°14), Familial Combined Hyperlipidemia (FCH) (n°15) or Undefined Autosomal Dominant Hypercholesterolemia (n°15), when they did not show the characteristics for FH or FCH. Children suffering chronic disease, diabetes or obesity were excluded. Children did not undergo any supplementary food or drug treatment since the last 3 months. Blood collection was performed on fasting state, when healthy since at least one month. Samples were stored at -80°C, until processed. Lipid profile parameters (TC, HDL-C, TG,) were assessed by automatic analyzer (Olympus AU 2700, Japan), LDL-C were calculated and OxLDL tested by enzyme linked immunosorbant assay (ELISA) (Mercodia AB, Uppsala, Sweden). Statistical analyses were performed using the SPSS 20.0 software (SPSS Inc, Chicago, IL).

Results. Lipid profile parameters in dyslipidemic and normocholesterolemic children were: TC 234±52.8 mg/dl, HDL-C 54± 13 mg/dl, TG 91 (42-195), LDL-C 162±51.8 mg/dl in the former group and TC 167.4±8.8 mg/dl, HDL-C 59±4.6 mg/dl, TG 49 (34-82), LDL-C 100.8±13.6 mg/dl in the latter group. The Ox-LDL level measurement resulted 67.97± 20.2 U/l in dyslipidemic children and 42.7± 5.2 U/l in normocholesterolemic children, this difference being statistically significant (p= 0.0001). As well a positive correlation was found between LDL-C and Ox-LDL in dyslipidemic children (p=0.003).

Conclusion. Increased Ox-LDL levels in children confirm an early exposure to oxidative stress that is detectable when primary dyslipidemia occur. These results underline the relevance of an early diagnosis to establish a primary prevention approach aimed to prevent the oxidative process.

PROBIOTICS SUPPLEMENTATION AND ANTIOXIDANT ACTIVITY IN HYPERCHOLESTEROLEMIC CHILDREN

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Introduction. Probiotics, as dietetic supplement, exert various biological roles on human health, one of the most debated being the antioxidant activity. This capacity may provide vascular defense against oxidative stress, implicated in the atherosclerosis progression. The aim of the study was to evaluate the effects of probiotic strains *Bifidobacterium lactis* BS05 and *Lactobacillus acidophilus* LA06 on the production of reduced glutathione (GSH) and superoxide dismutase (SOD), antioxidant parameters, in children affected by primary hyperlipidemia.

Material and Methods. A double-blind, randomized, placebo-controlled trial was performed on 44 hypercholesterolemic children, 10.4±2.8 years old, afferring to our department. They were randomized to receive probiotics or placebo for 3 months. Subjects were submitted to biochemical analyses at baseline and at the end of the treatment. Oxidized LDL (Ox-LDL), were tested by enzyme linked immunosorbant assay (ELISA) (Mercodia AB, Uppsala, Sweden) and glutathione levels (GSH and total glutathione)

by Biovision GSH Assay kit (Biovision). Lipid profile (TC, HDL-C, TG) was assessed by automatic analyzer (Olympus AU 2700, Japan) and LDL-C were calculated. Statistical analyses were performed using the SPSS 20.0.

Results. Lipid profile levels (mean±SD [mg/dl]: baseline TC 242.5±60, HDL-C 57.2±13.9, TG 92.8±33.8, LDL-C 167.6±60.6; after probiotics TC 227.7±47.0, HDL-C 56.0±11.8, TG 93.2±28.6, LDL-C 153.4±47.5) showed significant TC reduction (-6.0%, p=0.033) compared to baseline, with no significant difference between treatment and placebo. Ox-LDL and GSH were respectively 71.2±25.5 U/l and 27.1±6.5 ng/μL in basal conditions and decreased to 63.2±20.7 U/l (-9.0%, p=0.005) and 21.8±5.3 ng/μL (-18.8%, p=0.009) after probiotic treatment. These decrease were significant within-group but results were not significant compared with the placebo ones.

Conclusions. The effects of *Bifidobacterium lactis* BS05 and *Lactobacillus acidophilus* LA06 supplementation in children affected by primary hyperlipidemia ameliorate the Ox-LDL concentrations and worsen GSH levels. The contrasting results of probiotic effect, acting on GSH and SOD production need to be further investigated.

ANTI-INFLAMMATORY PROPERTIES OF APULIAN RED WINE POLYPHENOLS

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Red wine polyphenols have long been associated to a reduced morbidity and mortality from atherosclerotic coronary heart disease. Since wine polyphenolic content varies with cultivar, soil composition and climate aim of this study was to examine the antiatherogenic effects of polyphenolic extracts from two typical Apulian red wines, namely Primitivo and Negroamaro, by examining their biological effects on the activation of human endothelial and monocyte cells.

Methods. Human umbilical vein endothelial cells (HUVEC) and monocytes (U937) were treated with Primitivo and Negroamaro wine polyphenols (PWP and NWP) (10-80 μg GAEs/ml) or with pure polyphenols including trans-resveratrol (RSV), trans-piceid (RSVg), kaempferol (K), quercetin (Q), myricetin (M), caffeic acid (CAFF), caftaric acid (CAFT), coumaric acid (CUM) at 1-25 μmol/L for 60 min before stimulation with 20 nmol/L PMA or 2 mg/L LPS for 24 h. After treatments, we tested endothelial-monocyte adhesion and endothelial expression of adhesion molecules (VCAM-1, ICAM-1 and E-selectin) by enzyme immunoassay, the expression and activity of matrix metalloproteinases (MMP) by gelatin zymography both in activated endothelial and monocyte cells.

Results. Both PWP and NWP in a concentration dependent manner significantly inhibited the monocyte adhesion to endothelium, the endothelial expression of VCAM-1, ICAM-1, E-Selectin and the MMP-9 release and activity in HUVEC and U937, without any reduction in cell viability. PWP and NWP (80 μg GAEs/ml) inhibited endothelial and monocyte activation by about 40%. The same effect was obtained by 10 μM K, Q and RSV. However the amount of K, Q and RSV in PWP and NWP was equivalent only to 2.3% and 4% of PWP and NWP total polyphenols.

Conclusion. Polyphenolic extracts from Primitivo and Negroamaro red wines exhibit anti-atherogenic properties at very low concentrations. These effects were higher than those expected on the basis of individual polyphenol content suggesting a more than additive effect by different phenolic compounds.

OLIVE OIL POLYPHENOLS REDUCE VEGF-INDUCED ENDOTHELIAL CELL ANGIOGENIC RESPONSES BY CYTOPLASMIC AND MITOCHONDRIAL ROS INTERFERENCE

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Olive oil is a peculiar component of the Mediterranean diets which are associated with low incidence and prevalence of cardiovascular diseases and cancer. Inflammatory angiogenesis is a key pathogenic process both in cancer and atherosclerosis, and is tightly regulated by redox-sensitive pathways. We studied the effects of antioxidant polyphenolic extracts from virgin olive oil on endothelial cell angiogenic responses in vitro, and explored underlying mechanisms.

Purified polyphenols from Olive oil (OOP) were characterized by HPLC-UV-VIS as in (Gomez-Caravaca et al., Electrophoresis 2005) with minor modifications. Cultured endothelial cells from umbilical veins were pre-incubated with OOP (0-10 μg of gallic acid equivalents [GAEs]/mL) before stimulation with vascular endothelial growth factor (VEGF 25 ng/ml) for 16 hr. OOP significantly (p<0.05) reduced endothelial cell tube formation on matrigel and migration in wound healing assays with an IC₅₀ 2.5 μg GAEs/ml. By using specific pharmacological inhibitors, we found that VEGF-induced angiogenesis involved both cytoplasmic and mitochondrial-mediated pathways. The OOP-induced inhibition of angiogenic responses were accompanied by a significant reduction in the stimulated intracellular reactive oxygen species levels at both the cytoplasmic and mitochondrial compartments, as assessed by using 2',7'-dichloro-dihydrofluorescein and Mitosox Red assays, respectively.

Our findings reveal that olive oil polyphenols reduce VEGF-induced endothelial cell angiogenic responses through a reduction of ROS levels both at the cytoplasmic and mitochondrial compartments, supporting a potential protective role for olive oil polyphenols in atherosclerotic vascular disease and cancer.

CARDIOVASCULAR RISK FACTORS IN A POPULATION OF CHILDREN WITH SEVERE HYPERCHOLESTEROLEMIA: COMPARISON BETWEEN CHILDREN WITH AND WITHOUT MUTATION OF THE GENE CODING FOR LOW DENSITY LIPOPROTEIN CHOLESTEROL RECEPTOR

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Aim. To determine and compare a cardiovascular disease (CVD) risk profile, including classical (body mass index, TC, LDL-C, HDL-C) and emerging risk factors (ApoB, ApoA1, Lp(a), non-HDL-C) in children with and without genetic diagnosis of hetero-

zygous familial hypercholesterolemia (heFH) followed up at our Lipid Clinic.

Patients and Methods. 278 severely hypercholesterolemic children (median age 8.6 y, 134 male/144 female), at their first access to our Lipid Clinic, with positive family history for hypercholesterolemia and/or premature CVD, no ongoing pharmacological treatment, vitamin supplementation or secondary causes of hypercholesterolemia, were evaluated for: anthropometric measures, pubertal stage, twelve-hour fasting blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and Triglycerides (TGC) by enzymatic method, lipoprotein(a) (Lp(a)) levels by nephelometry, ApolipoproteinB (ApoB) and ApolipoproteinA1 by immunoturbidimetric assay and genetic analysis for heFH by polymerase chain reaction.

Statistics. Student's t test or Mann-Whitney test for independent samples.

Results. The 278 patients were divided in two groups: 137 had a mutation of the LDL-receptor (FH-group), 141 did not (non-FH-group).

Lipid profile (mg/dl, mean±ds) in the FH-group and in the non-FH-group was, respectively: TC 268.4±53 vs 213±46 (p<0.0001), LDL-C 194.4±55 vs 136.3±34. (p<0.0001), HDL-C 56.7±11.8 vs 59.2±13.6 (p=0.157), TGC 78±38.1 vs 74.9±33.7 (p=0.628).

The emerging risk factors (mg/dl, mean±ds) in the FH-group and in the non-FH-group were, respectively: ApoB 125.1± 34 vs 91.5±27 (p<0.0001), ApoA1 136.9±25 vs 141.3±23.4 (p= 0.116), Lp(a) 21.3±23.9 vs 29.6±36.8 (p=0.440), non-HDL-C 212.3±57.5 vs 153.5±46.1 (p<0.0001).

Conclusions. We found that children with genetic diagnosis of heFH present a worse CVD risk profile than hypercholesterolemic children without genetic diagnosis. Not only are TC and LDL-C higher in the FH-group, as described in previous studies, but also ApoB and non-HDL-C are higher in heFH children. These emerging risk factors have been recently introduced in pediatric research and are worth for further investigations.

EFFETTO A LUNGO TERMINE DI ALTE DOSI DI ACIDI GRASSI OMEGA-3 PER LA PREVENZIONE SECONDARIA DI EVENTI CARDIOVASCOLARI: UNA METANALISI DI TRIAL

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Background. Anche se le proprietà di riduzione del rischio di malattie cardiovascolari (CV) degli acidi grassi omega-3 sono ben documentate, l'evidenza da studi randomizzati e controllati (RCT) rimane inconcludente. È stata condotta una metanalisi degli RCT disponibili per indagare l'effetto di prevenzione cardiovascolare della somministrazione di almeno 1 g/die di omega-3 come supplemento per almeno 1 anno in pazienti con malattia CV pregressa.

Metodi. Sono stati ricercati RCT pubblicati fino a marzo 2013 in PubMed, EMBASE e Cochrane Library. Due autori hanno rivisto e selezionato gli studi ammissibili in modo indipendente.

Risultati. Di 360 articoli identificati, sono stati considerati nell'analisi 11 trial randomizzati, in doppio cieco e controllati con placebo che soddisfacevano i criteri di inclusione, coinvolgendo

complessivamente 15.348 pazienti con storia di malattia cardiovascolare. Non è stata osservata nessuna associazione statisticamente significativa tra l'assunzione di omega-3 e la mortalità per tutte le cause (risk ratio [RR] 0,89; IC 95% 0,78-1,02) o l'ictus (RR 1,31; 0,90-1,90). Al contrario, sono stati osservati effetti protettivi statisticamente significativi per la morte per cause cardiache (RR 0,68; 0,56-0,83), la morte cardiaca improvvisa (RR 0,67, IC 95%, 0,52-0,87) e l'infarto miocardico (RR 0,75; 0,63-0,88).

Conclusioni. Nel complesso, i risultati supportano l'ipotesi che l'effetto a lungo termine di alte dosi di omega-3 possa essere utile per prevenire l'insorgenza di morte cardiaca, morte improvvisa e infarto miocardico tra i pazienti con una storia di malattia cardiovascolare.

EFFECT OF TETRANECTIN-APOA-I INFUSIONS ON ATHEROSCLEROSIS PROGRESSION/REGRESSION IN HYPERCHOLESTEROLEMIC RABBITS

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Intravenous administration of synthetic HDL containing human apoA-I is effective in inducing atherosclerosis regression. A limitation of this therapeutic approach may be a rapid apoA-I turnover. Tetranectin-apoA-I, a trimeric human apoA-I, was designed to reduce renal clearance and thus prolong half-life and, possibly, efficacy. Aim of the study was to evaluate the effect of Tetranectin-apoA-I infusion on atherosclerosis in a rabbit model widely used to test the efficacy of synthetic HDL. Thirty-six rabbits underwent a perivascular injury at both carotids, followed by a 1.5% cholesterol diet. At 90 days after surgery, rabbits were randomly divided into 4 groups and i.v. treated, five times, once every three days, with different doses of Tetranectin-apoA-I (8, 40, 100 mg/kg) or with placebo. Plaque changes were evaluated in vivo by intravascular ultrasound (IVUS), performed before and at the end of the treatment period. Total atheroma volume in the placebo group increased in the time between the first and the second IVUS evaluation (+11.7±4.5% from baseline). A reduced progression or a regression was observed vs baseline in Tetranectin-apoA-I treated groups (+1.3±1.9% in 8 mg/kg; -0.03±4.80% in 40 mg/kg; -1.6±4.3% in 100 mg/kg treated rabbits). Absolute and percent changes of total atheroma volume in each Tetranectin-apoA-I group were significantly different from those found in the placebo group (p<0.005).

In conclusion, five Tetranectin-apoA-I administrations proved effective in reducing carotid plaque progression (8 and 40 mg/kg dose) or in inducing regression (100 mg/kg dose) in hypercholesterolemic rabbits.

EFFETTI DI NUTRACEUTICI IPOLEMIZZANTI SULLA PULSE WAVE VELOCITY, IN PAZIENTI IPERCOLESTEROLEMICI CON O SENZA PATOLOGIA RENALE

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La recente letteratura ha mostrato largo interesse sui nutraceutici ipolipemizzanti nel trattamento della colesterolemia moderata in soggetti con rischio di patologia cardiovascolare moderatamente elevato. L'obiettivo dello studio è stato quello di valutare gli effetti, a medio termine, sui parametri lipidici e sulla rigidità aortica con una terapia combinata con nutraceutici ipolipemizzanti.

Per lo studio sono stati arruolati 80 pazienti, tra cui: 40 con ipercolesterolemia moderata e patologia cronica renale da lieve a moderata e 40 pazienti ipercolesterolemici senza patologia renale cronica. Tutti i pazienti sono stati, giornalmente, trattati con un nutraceutico contenente riso rosso fermentato (3 mg di monacolina K) e berberina (500 mg). All'inizio del trattamento e dopo 6 mesi, sono stati misurati: pressione arteriosa, pulse wave velocity e parametri ematochimici. Nessun cambiamento significativo è risultato nei due gruppi per quanto riguarda il BMI, pressione arteriosa, livelli di transaminasi, creatin fosfochinasi, eGFR e metabolismo lipidico.

È stato riscontrato un miglioramento sia nei pazienti senza patologia renale di colesterolo totale (-21,6%), colesterolo LDL (-24,2%), Colesterolo nonHDL (-24,0%) e trigliceridi (-20,8%), sia nei pazienti con patologia cronica renale di colesterolo totale (-21,1%), colesterolo LDL (-23,7%), Colesterolo non HDL (-23,9%) e trigliceridi (-20,4%). I valori di PWV sono nettamente migliorati ($p < 0.01$) in tutti e due i gruppi senza differenze tra essi.

In conclusione, un trattamento con nutraceutici ipolipemizzanti, migliora sia il pattern lipidico che la pwv in pazienti con o senza patologia renale cronica.

ALTI LIVELLI DI ACIDO URICO SONO ASSOCIATI A RIDUZIONE DELLE FUNZIONI COGNITIVE IN PAZIENTI GIOVANI-ANZIANI: DATI DERIVATI DAL BRISIGHELLA HEART STUDY

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L'obiettivo dello studio è stato quello di valutare l'impatto dei livelli circolanti di acido urico sierico sulle funzioni cognitive, in una coorte di soggetti giovani-anziani non trattati farmacologicamente. Per questo studio di tipo trasversale, sono stati selezionati 288 pazienti dalle coorte storica del Brisighella heart Study (M: 108, F:180, con età 69 ± 6 anni), escludendo i pazienti trattati farmacologicamente, pazienti in prevenzione secondaria per patologie cardiovascolari, quelli con disordini neurovegetativi, con diabete confermato o gotta e quelli con aterosclerosi carotidee valutate ecograficamente.

Le funzioni cognitive sono state valutate tramite il Mini mental state (MMSE) e i dati sono stati analizzati tramite una regressione multipla con metodo stepwise, utilizzando un ampio set tra parametri di laboratorio e clinici, spessore miointimale carotideo e il Beck depression scale score.

L'analisi di regressione multipla ha mostrato come unici fattori associati al MMSE score: l'età ($B = -0.058$, 95% CI -0.108, -0.009, $p = 0.022$) e i livelli di acido urico sierico ($B = -0.527$, 95% CI -0.709, -0.344, $p = 0.022$). Ripetendo l'analisi in base al sesso è risultato che negli uomini sia l'età ($B = -0.151$, 95% CI -0.254, -0.048, $p = 0.005$) che i livelli di acido urico sierico ($B = -0.764$, 95% CI -1.142, -0.386, $p < 0.001$) sono associati al MMSE score, mentre nelle donne correlano inversamente solo i livelli di acido urico ($B = -0.339$, 95% CI -0.590, -0.087, $p = 0.009$).

In conclusione i livelli di acido urico sierico sembrano essere il maggior determinante della riduzione delle funzioni cognitive nel campione di soggetti giovani-anziani non trattati farmacologicamente.

PREDITTORI A LUNGO TERMINE DI RIDOTTA TOLLERANZA GLUCIDICA E DIABETE TIPO 2 IN SOGGETTI CON STORIA FAMILIARE DI DIABETE TIPO 2: 12 ANNI DI FOLLOW UP DELLA COORTE STORICA DEL BRISIGHELLA HEART STUDY

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L'obiettivo di questo studio è stato quello di quantificare il ruolo dei differenti fattori di rischio nello sviluppo a lungo termine di ridotta tolleranza glucidica e diabete di tipo 2, in un campione di popolazione rurale italiana con storia familiare di diabete di tipo 2.

Su un ampio campione di popolazione di 1851 pazienti, abbiamo selezionato 545 soggetti tra tutti quelli visitati durante il Brisighella Heart Study, escludendo quelli senza storia familiare positiva per il diabete tipo 2, quelli con ridotta tolleranza glucidica o diabete all'inizio dello studio e tutti quelli trattati con farmaci che possono interferire con il metabolismo glucidico.

È stata utilizzata un'analisi di regressione con metodo stepwise di Cox, per determinare il significato prognostico indipendente di un largo numero di parametri clinico-laboratoristici standard nell'insorgenza di diabete tipo 2 lungo 12 anni di follow-up.

Da questa analisi, appare come migliore predittore per l'insorgenza di diabete tipo 2 e ridotta tolleranza glucidica il livello di acido urico sierico, seguito da glicemia a digiuno (FPG), età, indice di steatosi epatica (HSI), pressione arteriosa media e colesterolo HDL.

Il modello di regressione di Cox che meglio predice l'incidenza di IFG e T2DM include età, sesso, FPG, TG e livelli di acido urico sierico; inoltre il modello di regressione di Cox che meglio predice l'incidenza di sola ridotta tolleranza glucidica è simile al precedente e include gli stessi parametri. Infine il modello di Cox che predice meglio l'insorgenza di diabete tipo 2 include FPG, BMI e HSI.

In conclusione possiamo affermare che in un campione di soggetti con storia familiare positiva per diabete i migliori predittori a lungo termine di ridotta tolleranza glucidica sono: età, genere, FPG, TG e SUA; mentre i predittori di diabete di tipo 2 FPG, BMI, ed HSI.

IL RISO ROSSO FERMENTATO MIGLIORA IL PATTERN LIPIDICO, I LIVELLI DI PROTEINA C REATTIVA AD ALTA SENSIBILITÀ E I PARAMETRI DI RIMODELLAMENTO VASCOLARE IN PAZIENTI ITALIANI CON IPERCOLESTEROLEMIA MODERATA

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Gli effetti di alti dosaggi di monacoline derivate dal riso rosso fermentato ed assunte come supplemento dietetico non sono state finora studiate su pazienti italiani. Il nostro scopo è stato quello di valutare se un trattamento a breve termine con 10 mg di monacoline potesse migliorare il pattern lipidico, i livelli di proteina C reattiva ad alta sensibilità e i parametri di rimodellamento vascolare in una piccola coorte di soggetti mediterranei, nell'ambito di un trial clinico, trasversale, randomizzato, in doppio cieco e controllato con placebo.

Per lo studio sono stati arruolati 25 soggetti sani, con ipercolesterolemia moderata, dopo 4 settimane di dieta stabile, i soggetti sono stati sottoposti, in maniera casuale, a una sequenza di trattamento: placebo-wash out- monacolina o monacolina-washout- placebo, ognuna di 4 settimane.

A ogni step dello studio sono stati misurati: pattern lipidico completo, parametri di salute, i livelli di proteina C reattiva ad alta sensibilità e le metalloproteasi della matrice (MMP) 2 e 9.

Quando confrontati con il gruppo di pazienti trattati con placebo, i trattati con monacolina, hanno mostrato un cambiamento percentuale migliore nel colesterolo totale (-12.45%, 95% CI -16.19 to -8.71), colesterolo LLL (-21.99%, 95% CI -26.63 to -17.36), non Hdl colesterolo (-14.67%, 95% CI -19.22 to -10.11), MMP-2 (-28.05%, 95% CI -35.18 to -20.93), P-9 (-27.19%, 95% CI -36.21 to -18.15), and hs-CRP (-23.77%, 95% CI -30.54 to -17.01). Nessuna differenza significativa invece è stata osservata in merito ai livelli di trigliceridi, HDL e parametri di sicurezza epatica e muscolare.

In conclusione, sulla base dei nostri dati, possiamo dimostrare che 10 mg di monacolina sembrano ridurre i livelli di colesterolo, hs-CRP ed i marker di rimodellamento vascolare in pazienti italiani.

Questi dati dovranno essere confermati in altri studi e in un campione di popolazione maggiore.

ASSESSMENT OF VASCULAR FUNCTION IN SIXTY HEALTHY MALES: SOCCERS SHOW IMPAIRED FEMORAL COMPLIANCE AND ENDOTHELIAL DYSFUNCTION

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Background. We evaluate effects of long lasting daily physical activity on morphology and function of carotid and femoral vessel wall, assessed in elite athletes aged 20 to 30 y.o. and age/sex-matched healthy controls.

Material and Methods. Thirty elite male athletes and 30 healthy male controls underwent medical examination, ankle brachial index, augmentation index (AIX), peripheral-arterial-tonometry (PAT), intima-media-thickness and pulse-wave-velocity assay at common carotid (c-IMT, c-PWv) and femoral arteries (f-IMT, f-PWv) by Doppler ultrasound.

Results. Athletes showed a significantly lower at rest heart rate (HR), and a better lipid profile.

In athletes c-PWv and f-PWv values (5.87 ± 0.80 m/sec and 6.62 ± 1.02 m/sec, $p=0.001$ and 8.96 ± 1.29 and 7.89 ± 1.39 , $p=0.002$, respectively) were, respectively, significantly lower and higher; carotid and femoral AIX were, respectively, lower (4.03 ± 6.21 vs 7.81 ± 5.21 , $p=0.003$) and higher (8.56 ± 10.21 vs 6.09 ± 7.95 ; $p=0.042$) in athletes. IMT values were significantly higher in controls (c-IMT: $p < 0.0001$; f-IMT: $p < 0.0001$). A positive significant correlation between HR and both c- f-IMT, respectively ($r=0.527$, $p < 0.0001$ and $r=0.539$, $p < 0.0001$, respectively) and between HR and c-PWv ($r=0.410$, $p=0.01$) were found when controls and athletes were considered as a whole group. Soccers showed lower PAT values in comparison to controls.

Conclusions. Elite sports positively affect c-IMT, f-IMT, carotid PWv and AIX, but not femoral PWv and AIX, and PAT. This behaviour could be due to prevalent involvement of muscular mass of inferior limb during exercise. Further studies are needed to understand whether this effect could limit the favourable effect of exercise in the control of cardiovascular risk.

IMPAIRMENT OF PERIPHERAL VASCULAR FUNCTION IN SYSTEMIC LUPUS ERYTHEMATOUS PATIENTS. DIFFERENCES FROM THE VASCULAR PATTERN OF PATIENTS AT HIGH CARDIOVASCULAR RISK

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Background. Growing evidence was collected that autoimmune diseases are associated to an enhanced atherosclerotic progression, and to a high incidence of cardiovascular events. Aim of our work was to investigate vascular wall function of peripheral arteries in Systemic Lupus Erythematosus (SLE) patients. Obtained results were then compared to those collected in patients with acute coronary syndromes (less 1-year previous event) and in healthy control subjects (no cardiovascular events or autoimmune disease).

Material and Methods. 170 patients (controls: 76, ACS: 74, SLE: 20), enrolled at Careggi Hospital, underwent to physical examination, fasting blood sampling and vascular function assessment of peripheral arteries: in particular, peripheral arterial tonometry (PAT) and augmentation index (Aix) by EndoPAT (Itamar, Caesarea, Israel), intima media thickness and pulse wave velocity at common carotid (c-IMT, c-PWv) and femoral arteries (f-IMT, f-PWv) by ultrasound technology (Esaote MyLab70), were assessed. The pattern of traditional cardiovascular risk factors (CRFs) was evaluated according to current guidelines (ADA, ATPIII, ESC, ESH, EAS guidelines).

Results. Study population resulted to be homogenous for age (SLE: 51.6 ± 11.3 ; ACS: 54.3 ± 8.1 ; controls: 49.7 ± 14.6 , $p=0.4$), but not for sex: 18/20(90%) of SLE patients were women. Among SLE

group, 8 (40%) had a previous vascular event (Venous thromboembolism: 3, ischaemic stroke: 4, acute coronary syndrome: 1).

ACS patients presented the worst cardiovascular profile, in comparison to others, because of a higher number of CRFs: ACS patients 3 ± 1.2 , SLE patients 1.5 ± 1.6 , controls 1.4 ± 1.3 . Therapies were performed according guidelines for secondary prevention.

ACS patients showed a marked atherosclerotic damage, because of higher c- and f-IMT values in comparison to others (ACS patients: c-IMT= 1.9 ± 0.6 mm, f-IMT= 1.7 ± 0.8 mm; SLE patients: c-IMT= 1.6 ± 0.8 mm, f-IMT= 1.5 ± 0.7 ; controls: c-IMT= 1.3 ± 0.9 mm, f-IMT= 1.2 ± 0.4); SLE and ACS patients data did not significantly differ, but both resulted to be significantly higher than data found in controls ($p=0.02$ and $p=0.03$, respectively).

Vascular compliance was significantly impaired in SLE patients (c-PWv= 10.5 ± 2.6 m/s, f-PWv= 10.2 ± 1.3 m/s), in comparison to ACS patients (c-PWv= 9.2 ± 2.3 m/s, f-PWv= 9.4 ± 3.1 m/s; $p<0.05$) and controls (c-PWv= 7.1 ± 2.1 m/s, f-PWv= 7.2 ± 1.4 m/s; $p=0.001$). Aix was higher in SLE ($17.2\pm 6.3\%$), in comparison to ACS patients ($15.3\pm 1.4\%$, $p=0.2$) and controls ($8.1\pm 4.2\%$; $p=0.002$).

SLE patients showed a significantly lower endothelial function, expressed as natural logarithm of reactive hyperaemia index (LnRHI), (0.56 ± 1.2), compared to controls (0.79 ± 1.7 ; $p=0.03$) and these values minimally differed between SLEs and ACS groups (0.50 ± 2.3 , $p=0.3$). LnRHI and PWv values were significantly correlated with number of CRFs. At univariate and multivariate analyses, presence of SLE and a previous vascular event were significantly associated to endothelial dysfunction ($p=0.02$ and 0.03 , respectively) and compliance impairment ($p=0.02$ and 0.03 , respectively).

Conclusions. Our data suggested that SLE patients showed a marked atherosclerotic peripheral vascular involvement, similar to patients at very high CV as ACS, because of impairment of local compliance at common carotid and femoral arteries and a marked endothelial dysfunction.

Despite the low cardiovascular risk profile, and the absence of a previous CV event in the 60% of subjects, SLE group showed a pattern of anatomical and functional damage of peripheral vessel as that found in patients at very high CV risk profile. These findings could contribute to the improve the early detection of the systemic and local vascular wall arterial damage in SLE patients and we hope they could contribute to the optimization of primary CV prevention in this high risk subgroup of patients.

EFFETTI ACUTI DELLA PROCEDURA DI LDL-AFERESI SULLA FUNZIONE ENDOTELIALE

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Introduzione. Scopo dello studio è stato valutare, in pazienti con Lp(a) >900 mg/L sottoposti a LDL-afèresi, gli effetti acuti della procedura aferetica sulla funzione endoteliale, indagata con: Tonometria Arteriosa Periferica (PAT, EndoPAT), Cellule Progenitrici Endoteliali (EPC) e Circolanti (CEC). Bassi valori di PAT ed EPC, e alti di CEC, si associano a disfunzione endoteliale e progressione aterosclerotica.

Materiali e Metodi. Sono stati studiati 8 pazienti [3 F e 5 M; età 64 (55-68) aa], sottoposti a cicli di LDL-afèresi (HELP (Plasmat Futura, B. Braun, Germany) ogni 7-10 giorni. Prelievi di sangue ve-

noso periferico (LDL, Lp(a); EPC, CEC) e PAT sono stati eseguiti al T0 (basale), T1 (post-procedura), T2 (24 ore); a 72 ore (T3) solo PAT, LDL e Lp(a). Le cellule progenitrici sono state identificate per l'espressione di CD34, KDR, e CD133, e considerate EPC se CD34+/KDR+, CD133+/KDR+ e CD34/CD133+/KDR+; CPC se CD34+, CD133+, e CD34+/CD133+. I valori di PAT, espressi come logaritmo naturale, erano patologici se $<0,40$.

Risultati. Al T0 nessun paziente presentava valori di PAT $<0,40$. Rispetto al T0, i valori di PAT erano inferiori al T1 ($-20,8\%$) e T2 ($-40,3\%$) (T0: $0,72\pm 0,2$, T1: $0,57\pm 0,2$, T2: $0,43\pm 0,2$, pT2 vs T0=0,03); tuttavia, al T3, superavano il T0 del $+54,2\%$ (T3: $1,11\pm 0,3$, pT3 vs T0=0,02; pT3 vs T1=0,01; pT3 vs T2=0,001), il T1, del $+94,7\%$, ed il T2, del $+158,1\%$.

Le EPC presentavano un progressivo incremento dal T0 al T2, in particolare Cd34+/Kdr+ (T0: $7,1\pm 11,8$; T1: $3,7\pm 4,8$; T2: $9,0\pm 5,47$) e Cd133+/Kdr+ (T0: $7,1\pm 10,8$; T1: $3,7\pm 4,8$; T2: $9,0\pm 5,8$). Le CEC, dopo un aumento al T1 ($30,4\pm 30,4$) erano significativamente ridotte al T2 ($12,0\pm 8,8$), rispetto al T0 ($23,8\pm 12,4$) (pT2vsT0=0,053).

L'incremento percentuale di PAT al T3vsT0 correlava direttamente con i livelli di Lp(a) al T0 ($r=0,342$, $p=0,04$) e T2 ($r=0,352$, $p=0,03$).

Conclusioni. La procedura si associa acutamente a disfunzione endoteliale (bassi livelli di PAT, alti di CEC), ma con un pronto recupero per valori di EPC e PAT, rispettivamente a T2 e T3, superiori al basale. Tali effetti positivi sulla funzione endoteliale, indagati per la prima volta acutamente, potrebbero rappresentare un effetto pleiotropico della procedura e associarsi al miglioramento del profilo lipidico nel ridurre la progressione aterosclerotica.

NON-ALCOHOLIC FATTY LIVER DISEASE AND SUBCLINICAL ORGAN DAMAGE: A NEW PREDICTOR OF ASYMPTOMATIC EXTRACORONARY ATHEROSCLEROSIS AND ENDOTHELIAL DYSFUNCTION

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Introduction. Intima-media thickness at common carotids (c-IMT) is a surrogate marker of extra-coronary atherosclerosis, and an independent predictor for cardiovascular mortality. Endothelial dysfunction by peripheral arterial tonometry (PAT), was associated to cardiovascular events, even in primary prevention subjects. Non-alcoholic fatty liver disease (NFLD) is known to be an independent risk factor for cardiovascular disease, associated to metabolic impairment facilitating progression of c-IMT, and also related with endothelial dysfunction; but scarce data are available about their relationship in primary prevention.

We investigated the predictive power of NFLD occurrence and severity in identifying extra-coronary atherosclerosis, evaluated by c-IMT and femoral IMT (f-IMT) measurement, and endothelial dysfunction, as reactive-hyperaemia index (LnRHI) values, in patients with no history of cardiovascular diseases.

Materials and Methods. Study population comprised 125 subjects (M:71; F:54; 45.1 ± 15.9 y.o.), without history of CV event or diabetes, who referred to our Center for vascular function assessment at ambulatory setting. Exclusion criteria were hepatitis B and C infection, alcohol consumption >30 g/day in men and >20 g/day in women, systemic diseases, and the use of drugs causing liver damage or fatty infiltration. All subjects underwent clinical assess-

ment for CV risk stratification, according to traditional risk factors (TRFs) and complete blood count and biochemical markers (lipid profile, transaminases and fasting glucose) were assessed. C-IMT, f-IMT, NFLD were investigated by ultrasound; and PAT. According to guidelines, c-IMT values >0.9 mm and f-IMT values >1.2 mm were considered pathologic; a natural logarithm of RHI (LnRHI) ≤ 0.40 was the cut off value for endothelial dysfunction. NFLD was expressed by a eight-point score, so that 0 defined absence and 8 maximum severity of steatosis.

Results. In this study population, 49% showed a c-IMT >0.9 mm, 40% a f-IMT >1.2 mm and 20% a LnRHI <0.4; regarding hepatic steatosis, 36% showed no liver involvement (score=0). Patients with impaired c-IMT were significantly older than others (46.6 ± 12.2 vs 56.4 ± 9.1 y.o.; $p=0.03$), showed a higher number of TRFs (1.7 ± 1.5 vs 3.2 ± 2.1 ; $p=0.02$), higher BMI (24.1 ± 3.6 vs 22.1 ± 2.7 ; $p=0.03$) and fasting glucose levels (0.95 ± 0.07 vs 0.89 ± 0.07 ; $p=0.001$) in comparison to others; we also found a more marked prevalence of hypertensives ($n=18\%$; $p=0.04$), although all patients were in good pharmacological control. No difference regarding prevalence of dyslipidaemia. Significant positive correlations between steatosis score, fasting glucose ($r=0.391$, $p=0.03$), and BMI ($r=0.423$, $p=0.01$) were found. C-IMT >0.9 mm was significantly associated to a more marked severity of hepatic steatosis (5.1 ± 2.2 vs 2.4 ± 1.7 ; $p<0.0001$) and to a lower endothelial function (0.41 ± 0.07 vs 0.9 ± 0.12 ; $p=0.001$); specifically, f-IMT >1.2 mm was significantly associated to higher steatosis score (4.9 ± 2.3 vs 2.5 ± 1.9 ; $p=0.001$). ROC curve analyses for steatosis score showed higher AUC values in discriminating c-IMT >0.9 mm (AUC 0.76, 95%CI 0.67-0.84, $p<0.0001$) and f-IMT >1.2 mm (AUC 0.73, 95%CI 0.65-0.81, $p=0.0002$), compared ROC of TRFs, respectively for c-IMT (AUC 0.70, 95%CI 0.62-0.79, $p=0.01$) and f-IMT (AUC 0.69, 95%CI 0.61-0.77, $p=0.027$). Regarding the prediction of endothelial dysfunction, ROC curve analyses for steatosis score showed higher AUC values in discriminating patients with LnRHI <0.4 (AUC 0.80, 95% CI 0.72-0.87, $p<0.0001$), in comparison to ROC of TRFs (AUC 0.74, 95% CI 0.65-0.85, $p=0.0001$). At univariate analysis, a >3 steatosis score was significantly associated to c-IMT >0.9 mm ($p<0.0001$), f-IMT >1.2 mm ($p=0.001$) and endothelial dysfunction ($p>0.0001$), even after correction for TRFs at multivariate analyses.

Conclusions. Our study provides evidences that NFLD occurrence and severity may select patients with endothelial dysfunction and extra-coronary atherosclerosis, in a primary prevention population, independently of traditional cardiovascular risk profile. A 3 value of NFLD score may be considered as cut-off of clinical relevance.

ECHOCARDIOGRAPHY ASSESSMENT OF VALVULAR HEART DISEASE IN FAMILIAL HYPERCHOLESTEROLEMIA

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Premature and accelerated atherosclerosis, with clinical signs of cardiovascular disease (CVD) and frequent involvement of left heart valves, resulting in stenosis and/or incompetence, is a feature of patients with homozygous Familial Hypercholesterolemia

(FH). The incidence of aortic stenosis is lower in heterozygous FH without other cardiovascular risk factors. The aim of this study was to evaluate the impact of LDL levels on aortic and mitral valve disease in FH.

Materials and Methods. Baseline levels of LDL (before starting lipid-lowering therapy) were determined in 66 patients (56.1% women; 44 ± 14 years) with genetic diagnosis of FH, without other metabolic disorder. In our sample have been identified 22 different mutations of LDLR gene in heterozygosis, 4 in compound heterozygosis and 1 in homozygosis. All patients underwent transthoracic echocardiogram with 2D and Doppler images. The degree of aortic valve calcification and mitral annular calcification (MAC) was semi-quantitated from absent to severe following a standardized protocol. Patients were defined having higher LDL, when their LDL were higher than the median value (303 mg/dl) in the whole population sample.

Results. The prevalence of CVD was significant higher in patients with higher LDL compared to those with lower LDL (35% vs 12%), independently of age ($p<0.031$). Prevalence of aortic stenosis and severity of mitral or aortic valve calcification was higher in these patients, independently of age, gender, BMI and hypertension ($p=0.048$; OR=5,73; 95% I.C. 1,01-32,35). The same analysis was at the limit of statistical significance when adjusted for history of CVD ($p=0.06$).

Conclusion. In a FH population the degree of aortic valve calcification and MAC has been suggested as a surrogate for premature CVD. Further studies are needed to evaluate the association of specific mutations with aortic and mitral valve abnormalities.

RELAZIONI TRA IPOVITAMINOSI D, APOLIPOPROTEINE ED ATEROTROMBOSI IN PAZIENTI CON PAD

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Introduzione. Dati provenienti da numerosi studi epidemiologici suggeriscono una forte associazione tra ipovitaminosi D e malattie cardiovascolari. Recenti evidenze sembrano dimostrare come l'ipovitaminosi D sia associata ad un profilo di rischio cardiovascolare meno favorevole. Si è dimostrato infatti come l'ipovitaminosi D sia associata all'ipertensione arteriosa, all'obesità, al Diabete mellito, ed a valori bassi di HDL. Pochi dati, peraltro contrastanti sono disponibili sulle associazioni presenti tra ipovitaminosi D, apolipoproteine e fattori protrombotici. Lo scopo del nostro studio è stato quello di valutare le relazioni esistenti tra livelli circolanti di vitamina D 25 OH, Apolipoproteine e markers di aterotrombosi in una popolazione selezionata di 150 pazienti affetti da arteriopatia periferica in cui notoriamente si riscontrano frequentemente livelli ridotti di Vitamina D.

Materiali e Metodi. Nel nostro studio sono stati inclusi 150 pazienti consecutivi ricoverati presso la Clinica Medica dell'Azienda Ospedaliera Universitaria "S. Maria della Misericordia" di Udine con diagnosi clinica e strumentale di arteriopatia obliterante cronica degli arti inferiori. Tutti i pazienti inclusi nello studio sono stati sottoposti a prelievi ematici per analisi effettuate presso il Laboratorio Analisi dell'Azienda Ospedaliero-Universitaria di Udine con metodo standardizzato.

Le variabili continue sono state comparate con il metodo statistico ANOVA (analisi della varianza. Per le variabili che non presentavano una distribuzione normale, gli studi statistici sono stati effettuati sui rispettivi logaritmi. Le variabili categoriche sono state invece studiate con il metodo Chi-Quadrato e sono state espresse

in numeri assoluti e percentuali. Al fine di valutare la forza ed il tipo dell'associazione delle variabili sono stati valutati gli indici di correlazione di Pearson e la regressione lineare univariata tra livelli di vitamina D 25 OH ed ApoB, ApoA1 e B/A ratio oltre che con i marker di aterotrombosi (d-dimero, fibrinogeno, F1+2, ATIII, Proteina C ed S, APC-r). È stato quindi costruito un modello per l'analisi multivariata con metodica "Stepwise Multiple Linear Regression" (variabili introdotte: Età, BMI, GFR, Colesterolemia totale, LDL, Apolipoproteina B, B/A ratio, Fibrinogeno, F1+2, VES, Log PCR, emoglobina glicata) aggiustato per confondenti quali fumo e presenza di diabete mellito, per valutare quali fossero le variabili indipendentemente associate al deficit di Vitamina D 25 OH. Per tutti i test è stato considerato significativo un P-value <0,05.

Risultati. Dal'analisi dei dati sono emerse significative correlazioni negative tra Vitamina D 25 OH ed B/A ratio, F1+2, Fibrinogeno e D-Dimero.

All'analisi multivariata le variabili risultate essere indipendentemente associate a ridotti livelli di Vitamina D 25 OH sono risultate essere B/A ratio ($r=0,178$ $p=.05$) e F1+2 ($r=0,295$ $p=.001$). Il frammento aminoterminale della protrombina si produce in seguito alla trasformazione della protrombina in trombina ad opera dell'enzima protrombinasi nella fase della coagulazione piastrinica, livelli elevati in circolo esprimono in sostanza un'esaltata attivazione della coagulazione. È ragionevole quindi supporre che la vitamina D possa esercitare in qualche maniera un ruolo nella regolazione dell'attività della protrombinasi. Tale effetto potrebbe esplicarsi attraverso l'influenza della vitamina D sulla sintesi del fosfolipide di membrana fosfatidiletanolamina. E.H Wasserman già nel 1982 aveva dimostrato come supplementi di vitamina D fossero in grado di inibire l'espressione di tale fosfolipide è quindi ragionevole supporre che ridotti livelli di vitamina D si associno ad elevata sintesi dello stesso. Recentemente Rinku Majumder et al hanno dimostrato un ruolo importante della fosfatidiletanolamina nella regolazione dell'attività della protrombinasi, in particolar modo si è dimostrato come essa sia in grado di attivare l'enzima che converte la protrombina in trombina in maniera indipendente. Il ragionamento che ne deriva è che ridotti livelli di vitamina D possano incrementare la sintesi di fosfatidiletanolamina e conseguentemente venga esaltata l'attività della protrombinasi. Questi dati (che andranno confermati con studio ad Hoc) suggeriscono un ruolo della vitamina D sulle variazioni del profilo trombotico e sulla possibilità che un suo deficit possa favorire la realizzazione di uno stato trombofilico e l'insorgenza/progressione di patologie aterosclerotiche quali la PAD.

STUDY OF POLYGENIC FAMILIAL HYPERCHOLESTEROLEMIA IN SOUTHERN ITALY

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Introduction. Familial Hypercholesterolemia (FH) is the most common form of autosomal dominant hypercholesterolemia. The LDL receptor (LDLR) gene is the locus mainly involved in FH while the Apolipoprotein B (APOB) and Proprotein Convertase,

Subtilisin/Kexin-type 9 (PCSK9) genes are involved in a lower percentage of cases.

A recent study in English and Belgium FH patients (Talmud et al Lancet 2013), has reported that in a proportion of patients where no mutation can be found in these genes, that the disease could be polygenic, due to SNPs strongly associated with low-density lipoprotein cholesterol (LDL-C).

Materials and Methods. We enrolled 199 patients with clinically diagnosed FH, of whom 160 were unrelated. The LDLR, ApoB, PCSK9 genes were amplified by PCR and directly sequenced. The TaqMan assay was performed, in all the FH patients and in 3,020 controls from the UK Whitehall II (WHII) study, for 12 common SNPs that the Global Lipid Genetic Consortium (GLGC) reported as significantly associated with LDL-C. For each samples, we calculated LDL-C specific gene scores using the weighted sum of the risk allele.

Results. The screening revealed mutations in 141 patients. In 58 patients with a clinical diagnosis of FH but no detected mutation, the mean 12 SNP LDL-C gene score was 0.96 (SD 0.17) which was significantly higher than 0.90 (SD 0.23) for the WHII study ($p=0.0046$). By contrast, in the 141 FH patients with a detected mutation the mean weighted score was 0.93 (SD 0.21), showing a trend of significant difference with the score of WHII ($p=0.057$).

Conclusions. These results confirm the previous report, and show that, also in Italian patients with the FH phenotype but without mutations in the main candidate genes, there is a likely polygenic cause, due to the inheritance of LDL-C-raising SNPs which increases LDL-C concentration in patients. This polygenic contribution is also seen in patients with detected FH-causing mutations.

EFFETTI DEL CACAO SULLA FUNZIONE ENDOTELIALE E SULLA REATTIVITÀ DEL CIRCOLO CEREBRALE DURANTE TEST COGNITIVI

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Introduzione. L'effetto positivo del cacao sul tono dell'umore e sulla funzione vascolare è stata documentata in letteratura, mentre meno chiari risultano gli effetti sulla reattività neuro-vascolare durante attivazione (linguaggio e calcolo); tale aspetto potrebbe avere rilievo nel trattamento di patologie involutive e degenerative. Lo scopo del nostro studio è stato quello di valutare come il cacao possa migliorare il flusso cerebroaffente misurato con ecodoppler transcranico durante un test linguistico (TL) e durante un test matematico (TM) in soggetti sani.

Pazienti e Metodi. 12 soggetti sani (29-42 aa) sono stati sottoposti a misura del calibro e flusso dell'arteria carotide interna bilateralmente e a calcolo della velocità media del flusso sull'arteria cerebrale media d'ambo i lati, prima e durante TL (traduzione dall'inglese, 3 minuti) e TM (conto alla rovescia 3 minuti). Abbiamo inoltre misurato la vasodilatazione endotelio-mediata all'arteria omerale destra mediante metodica ultrasonografica e test post-ischemico all'avambraccio sinistro. Questi test sono stati ripetuti dopo 90 minuti dalla somministrazione di cacao commerciale in polvere (50 g =15-20 mg circa di epicatechina) diluito in 30 cc di acqua tiepida.

Risultati. Entrambi i test cognitivi hanno determinato un lieve incremento, non significativo, sia dei valori pressori diastolici che della frequenza cardiaca. L'assunzione di cacao non ha determinato

significative modificazioni dei valori pressori. Per quanto riguarda il flusso carotideo abbiamo registrato un incremento significativo al basale durante TL a sinistra ($48,5 \pm 2,4$ vs $40,2 \pm 3,1$ cm/s, $p < 0,05$); la somministrazione di cacao ha evidenziato un significativo incremento in corso di TM a sinistra ($13,2 \pm 3,2$ vs $3,6 \pm 2,4$; $p < 0,05$) e durante TL bilateralmente con maggior rilievo a sinistra ($46,5 \pm 4,6$ vs $15,2 \pm 3,8$; $p < 0,005$). Per quanto riguarda la velocità di flusso sulla cerebrale media, questa è incrementata sia durante TM a sinistra ($60,2 \pm 3,3$ vs $54,3 \pm 1,4$ cm/s; $p < 0,05$) che durante TL sempre a sinistra ($62,6 \pm 3,4$ vs $59,1 \pm 1,1$ cm/s; $p < 0,05$). Dopo cacao, la velocità di flusso sulla cerebrale media incrementa durante TL a sinistra ($11,3 \pm 2,1$ vs $7,2 \pm 3,5$; $p < 0,05$) e durante TM bilateralmente (a dx $5,8 \pm 2,2$ vs $1,2 \pm 0,5$; $p < 0,05$ - a sn $7,5 \pm 2,6$ vs $3,6 \pm 2,1$; $p < 0,05$). Il diametro delle arterie carotidi non ha mostrato variazioni durante i test e neppure dopo somministrazione di cacao. Lo studio della vasodilatazione endotelio-mediata ha evidenziato un incremento dopo somministrazione di cacao ($11,4 \pm 2,1$ vs $8,9 \pm 3,8$; $p < 0,05$).

Discussione. Nel nostro studio documentiamo un miglioramento della risposta emodinamica del circolo cerebroaffente ai test cognitivi dopo somministrazione di cacao; evidenziamo inoltre un miglioramento della funzione endoteliale. La maggiore attivazione emodinamica riscontrata dopo cacao potrebbe essere ascrivibile agli effetti favorevoli che i contenuti di tale alimento determinano sulla funzione endoteliale. Una maggiore attività del tessuto cerebrale potrebbe essere inoltre invocata, anche se dalla letteratura non emergono dati univoci. La lateralizzazione di una buona parte dei risultati a sinistra corrisponde ovviamente alle aree cerebrali che vengono elettivamente attivate; il cacao sembra tuttavia esaltare la bilateralità di alcune risposte a TM. I risultati del nostro studio, seppur in forma di dati preliminari, possono far ipotizzare studi con più ampia casistica per valutare il possibile ruolo di questo alimento o di sue componenti, nella prevenzione o terapia di patologie neurodegenerative.

COMORBIDITÀ E DETERMINANTI DI BASSI LIVELLI DI C-HDL IN PAZIENTI OSPEDALIZZATI CON REAZIONE DI "FASE ACUTA"

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Background. È dimostrato in popolazioni selezionate che la reazione di fase acuta è caratterizzata da alterazioni nel profilo lipidico: riduzione del colesterolo totale, HDL-C e LDL-C, con trigliceridemia normale/aumentata.

Scopo dello Studio. Valutare le differenze del profilo lipidico in pazienti ospedalizzati con o senza reazione di fase acuta.

Pazienti e Metodi. sono stati inclusi 902 pazienti (età 20-80 anni) suddivisi sulla base dei valori di PCR al momento del ricovero: gruppo 1 (PCR $\geq 1,5$ mg/dl) e gruppo 2 (PCR $< 0,5$ mg/dl). Abbiamo riclassificato la popolazione in sottogruppi sulla base della diagnosi formulata alla dimissione secondo ICD-9-CM: infezioni, malattie reumatiche, neoplasie, malattie cerebrovascolari, traumi e disordini mentali, malattie endocrino-metaboliche e soggetti senza significative morbidità. Parametri determinati: PCR, globuli bianchi, alfa-2 globuline, emoglobina, piastrine, colesterolo totale, HDL-C, LDL-C, trigliceridi, glicemia, creatinina, transaminasi, albumina.

Risultati. I soggetti con PCR $\geq 1,5$ mg/dl sono più anziani ($p < 0,001$) e con livelli più elevati di leucociti ($p < 0,001$), alfa-2 globuline ($p < 0,001$), glicemia ($p < 0,001$), e con valori più bassi di HDL-C ($p < 0,001$) e LDL-C ($p < 0,001$). Solo il sottogruppo "infezioni" ha mostrato una PCR significativamente più elevata e valori di HDL-C ed LDL-C significativamente più bassi rispetto agli altri sottogruppi. Sia HDL-C che LDL-C hanno mostrato una correlazione inversa con rispettivamente: PCR ("p"-.402, $p < 0,001$; "p"-.294, $p < 0,001$), leucociti ("p"-.254, $p < 0,001$; "p"-.169, $p < 0,001$), alfa-2 globuline ("p"-.110, $p = 0,001$; "p"-.025, $p = 0,462$). Alla regressione multivariata sesso ("β"-.322, $p < 0,001$), albumina ("β".255, $p < 0,001$), trigliceridi ("β"-.219, $p < 0,001$) e PCR ("β"-.126, $p = 0,001$) sono predittori indipendenti di HDL-C (R.285, $p < 0,001$).

Discussione. Questi dati estendono l'osservazione della riduzione di HDL-C e LDL-C durante fase acuta ad una popolazione di soggetti più eterogenea rispetto ai precedenti studi. La maggior riduzione di HDL-C si è osservata nei pazienti appartenenti al sottogruppo "infezioni". La PCR è l'unico parametro di fase acuta in grado di predire la riduzione dei valori di HDL-C.

RISK FACTORS FOR CAROTID ARTERY ATHEROSCLEROSIS IN A CARDIAC INTENSIVE UNIT INCIDENCE OF HEMODYNAMICALLY SIGNIFICANT CAROTID ARTERY STENOSIS IN PATIENTS ADMITTED TO CARDIOVASCULAR INTENSIVE CARE UNIT. WHAT ARE THE RISK FACTORS?

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Introduction. Several studies have assessed the relationship between the presence of atherosclerosis in carotid arteries and the risk of coronary artery disease (CAD) or cardiovascular events. Moreover the coexistence of carotid disease is considered as one of the avoidable sources of the occurrence of stroke during coronary artery bypass grafting (CABG) with age > 70 years old, smoke, peripheral artery disease (PAD), previous transient ischemic attack (TIA) or stroke and neck bruit. Aim of our study was to evaluate the incidence of carotid artery disease (CVD) and the relation between hemodynamically significant carotid artery stenosis and admission diagnosis, cardiovascular risk factors and coronary lesions in patients admitted to the Cardiovascular Intensive Care Unit (CICU) of the University of Florence in order to establish a predictive model of selection of patients to submit to carotid screening.

Methods. We prospectively studied patients admitted to our CICU from January to December 2012 with diagnosis of stable angina, acute coronary syndrome, myocardial infarction, coronaropathy and arrhythmias. Duplex of neck arteries have been systematically performed in all patients by using a 7.5 MHz linear probe (Philips Sonos 5005).

Results. We screened 367 consecutive patients admitted to CICU. Twenty-nine patients were excluded from the study owing to an history of neurological disorders (stroke or TIA). Among these 338 subjects, 91 patients had no lesions and 262 (77.5%) presented at least a $> 50\%$ stenosis lesion (systolic peak velocity $> 1,5$ m/s, end-diastolic velocity $> 0,5$ m/s and peak systolic internal carotid/common carotid ratio $> 2,2$). In 204 patients (82,5%) internal or bulb ca-

rotid were involved. No patient had vertebral arteries involvement. Fifteen (4,4%) had >70% stenosis lesion (systolic peak velocity >2.5 m/s, end-diastolic velocity >1 m/s and peak systolic internal carotid/common carotid ratio >3).

We compared patients with and without lesions in relation to morbidity and risk factors: age ($p<0.001$), hypertension ($p<0.001$), mellitus diabetes ($p<0.001$), PAD ($p=0.006$), chronic obstructive pulmonary disease ($p=0.008$) and value of serum creatinine at admission ($p=0.02$) were predictive of the presence of significant CVD.

Among patients with >70% stenosis, 10 were submitted to coronarography: 6 (60%) had a three vessels disease, 2 (20%) a two vessels disease, 2 (20%) a single vessel disease, no patients had no lesions. The extend of coronary involvement is a significant predictor of significant stenosis (OR: 2.04; CI 95%: 0.91-4,68; $P=0.084$).

There is no relation between the presence of >70% stenosis lesion and admission diagnosis.

Univariate analysis showed that the presence of >70% stenosis lesion is related to hypertension ($P=0.058$), chronic kidney disease (CKD) ($P=0.020$) and PAD ($P<0.001$). Multivariate analysis further recognized PAD as a significant predictor for >70% stenosis (OR:10.2; CI 95%:3.16-33.0; $P<0.001$) when corrected for hypertension (OR: 2.62; CI 95%: 0.55-12,5; $P= 0.226$) and CKD (OR: 1.80; CI 95%: 0.48-0.74; $P=0.383$).

Conclusion. Our data revealed that the presence of >70% stenosis lesion increased risk of early death in patients admitted to CICU. Moreover carotid lesions were related to extend of coronary involvement. Patients with CKD, hypertension and PAD had higher risk for >70% stenosis lesion.

VARIAZIONE DI ALCUNI PARAMETRI METABOLICI E DI ALCUNE ADIPOCITOCHINE DOPO LA SOMMINISTRAZIONE DI UN ESTRATTO DI BERBERIS ARISTATA/SILYBUM MARIANUM IN PAZIENTI SOVRAPPESO E DISLIPIDEMICI

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Scopo. Valutare gli effetti di una combinazione di estratto di Berberis Aristata/Silybum Marianum in un campione di soggetti in sovrappeso e dislipidemici a basso rischio cardiovascolare.

Materiali e Metodi. Sono stati arruolati 105 pazienti caucasici, in sovrappeso, dislipidemici. Dopo un periodo di 6 mesi di run-in durante il quale è stato seguito un regime dietetico e praticata attività fisica, i pazienti sono stati randomizzati ad assumere placebo o una combinazione di estratto di Berberis aristata/Silybum marianum 588/105 mg, 1 compressa a pranzo e una compressa a cena, per tre mesi. Berberis aristata/Silybum marianum e placebo sono stati, poi, interrotti per 2 mesi e ripresi per ulteriori 3 mesi. Abbiamo valutato i parametri antropometrici, la glicemia a digiuno, l'insulinemia a digiuno (FPI), il profilo lipidico, l'indice HOMA di resistenza insulinica (HOMA-IR), la proteina-4 legante il retinolo (RBP-4), l'adiponectina (ADN) e la resistina.

Risultati. Il colesterolo totale ($p<0,01$), il colesterolo LDL ($p<0,01$) e i trigliceridi ($p<0,05$) si sono ridotti e il colesterolo HDL ($p<0,05$)

è aumentato dopo 3 mesi di Berberis aristata/Silybum marianum, rispetto alla randomizzazione e a placebo. Berberis aristata/Silybum marianum ha ridotto il valore di FPI e l'HOMA-IR, sia rispetto al basale che a placebo ($p<0,05$ per entrambi). C'è stata, inoltre, una riduzione di RBP-4 e resistina e un aumento di ADN dopo 3 mesi di Berberis aristata/Silybum marianum. Tutti questi effetti sono scomparsi durante il periodo di wash-out e sono ricomparsi dopo la ripresa di Berberis aristata/Silybum marianum. È stata osservata una correlazione significativa tra riduzione dell'HOMA-IR e riduzione di resistina e RBP-4, e tra riduzione di HOMA-IR e aumento di ADN nel gruppo trattato con Berberis aristata/Silybum marianum.

Conclusioni. L'associazione di Berberis aristata/Silybum marianum è efficace nel migliorare non solo il profilo lipidico, ma anche l'insulino-resistenza.

GAMMA-GLUTAMILTRANSFERASI E RIGIDITÀ DELLA PARETE ARTERIOSA NEL GRANDE OBESO

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Presupposti. Diversi studi epidemiologici hanno evidenziato l'esistenza di una correlazione fra livelli plasmatici di gamma-glutamyltransferasi (GGT) e mortalità cardiovascolare. La steatosi epatica rappresenta una causa comune di elevazione dei livelli plasmatici di GGT fra i soggetti obesi e si associa ad una maggiore rigidità della parete arteriosa.

Scopi. Valutare, in una coorte di grandi obesi, la relazione fra steatosi epatica, GTT e rigidità della parete arteriosa.

Materiali e Metodi: 90 soggetti grandi obesi (BMI-medio: 44 kg/m²) di età compresa fra i 18 ed i 62 anni. Indagini effettuate: anamnesi, esame obiettivo, parametri antropometrici ed esami ematochimici. Grading della steatosi epatica con metodica ecografica. Misurazione della pulse wave velocity aortica (aPWV), indice indiretto e non invasivo della rigidità arteriosa, mediante tonometro ad applanazione.

Risultati. La prevalenza della steatosi epatica nella popolazione studiata era del 91%.

I soggetti con steatosi epatica moderata/severa presentavano livelli plasmatici di GGT significativamente più elevati rispetto ai soggetti con steatosi lieve/assente (47,4 UI/L vs 27 UI/L, $p<0,01$). La aPWV è risultata essere correlata con l'età ($p<0,01$), la pressione arteriosa sistolica ($p<0,05$), la GGT ($p<0,05$), la trigliceridemia ($p<0,05$) e la glicemia ($p<0,05$).

Nella analisi multivariata l'età ed i livelli plasmatici di GGT sono risultati essere predittori indipendenti della aPWV (altre variabili incluse nel modello erano: sesso, fumo, pressione arteriosa sistolica, trigliceridi e glicemia).

Discussione. I livelli plasmatici dell'enzima GGT sono risultati essere predittori indipendenti della rigidità della parete arteriosa. L'incremento dei livelli di GGT potrebbe essere uno dei meccanismi che spiegano l'associazione fra steatosi epatica ed irrigidimento della parete arteriosa.

L'irrigidimento della parete arteriosa rappresenta un importante predittore indipendente di mortalità cardiovascolare, pertanto la determinazione dei livelli plasmatici di GGT nei soggetti grandi obesi può fornire utili informazioni aggiuntive per una più accurata stratificazione del rischio cardiovascolare.

GENETIC SCREENING OF PAEDIATRIC PATIENTS SUFFERING FROM FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Familial Hypercholesterolemia (FH) is a severe monogenic hyperlipidemia leading to very high levels of LDL cholesterol, associated with increased cardiovascular risk and in some cases with the presence of tendinous xanthomatosis. The early identification of FH patients can be useful for establishment of an adequate therapy and the prevention of cardiovascular accidents. We are reporting the results of a genetic screening performed in a paediatric population.

Patients and Methods. Sixty-three unrelated patients under 16 years (mean age \pm SD=9.8 \pm 3.6 years) were enrolled during 2012-2013 in different Italian clinics. In 55 patients the LDLR screening was performed; 53 were clinically diagnosed as possible and 2 as definite FH based on the Simon Broome criteria. The promoter and 18 exons of the LDLR gene were amplified by PCR and directly sequenced. MLPA was performed to identify large rearrangements.

Results. Direct sequencing analysis and MLPA revealed mutations in the LDLR gene in 46/55 unrelated FH patients (mutation rate 83.6%). Two patients are compound heterozygotes (3.6%) and 44 are heterozygotes (80%). The 2 patients with a more severe phenotype leading to a definite diagnosis are a compound heterozygote and a heterozygote. The mutation found are 30 missense (54.5%), 8 splicing (14.5%), 4 nonsense (7.3%), 1 duplication (1.8%), 1 small deletion (1.8%) and 2 large deletions (7.3%). Among heterozygote patients the presence of a radical mutation (splicing, nonsense, duplication and deletion) leads to higher values of LDL cholesterol (252 \pm 52 mg/dL) respect to carriers of missense mutations (198 \pm 24 mg/dL) with $p=0.001$.

Conclusions. The screening for LDLR mutations showed a high mutation rate in paediatric patients, although their diagnosis was predominantly not definite because the lack of xanthomatosis due to the young age. Carriers of radical mutations showed a severe lipid phenotype since childhood suggesting a strict follow up and an early initiation of therapy.

STIMA DELLA FREQUENZA DI MUTAZIONI DEL GENE LDLRAP1 NELLA POPOLAZIONE SARDA

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Background. L'ipercolesterolemia autosomica recessiva è un disordine monogenico presente con frequenza relativamente elevata in Sardegna (1:40.000). Due mutazioni, G65A (W22 \rightarrow stop) situata

nell'esone 1 del gene LDLRAP1 e 432insA (E170 \rightarrow stop) situata nell'esone 4 dello stesso gene sono responsabili di tutti i casi finora riportati. È stato effettuato uno screening di tali mutazioni per la stima della frequenza degli eterozigoti in un campione di popolazione sarda. Sono stati sottoposti ad analisi molecolare 2448 campioni di DNA rappresentativi della popolazione del Nord e Sud Sardegna.

Metodi. L'analisi è stata effettuata mediante amplificazione PCR del primo e del quarto esone del gene LDLRAP1 con primers mutagenizzati, seguita da analisi di restrizione (rispettivamente BstNI e MvaI per le mutazioni nell'esone 1 e 4). Per la mutazione G65A il primer reverse è stato modificato nella penultima base all'estremità 3' per creare un nuovo sito di restrizione BstNI, mentre per la mutazione 432insA il primer generava un nuovo sito di restrizione MvaI. Nel caso della mutazione G65A in presenza dell'allele mutato viene eliminato il sito di restrizione e si osserva la banda dell'amplificato di 157 bp. Nel caso della mutazione 432insA in presenza dell'allele mutato viene eliminato il sito di restrizione e si osserva la banda dell'amplificato di 144 bp.

Risultati. Sono stati riscontrati 3 eterozigoti su 2448 (0,122%) per la mutazione G65A e 16 eterozigoti su 2448 (0,653%) per la mutazione 432insA. Inoltre è stata riscontrata la presenza di un omozigote su 2448 (0,040%) per la mutazione 432insA. Dai dati suddetti la stima della frequenza degli eterozigoti nella popolazione sarda risulterebbe essere di 1:820 per la mutazione G65A e 1:153 per la mutazione 432insA.

Conclusioni. La prevalenza osservata per la mutazione 432insA non si discosta significativamente da quella attesa (1:135), stimata sulla base della frequenza degli omozigoti nella popolazione generale. Al contrario la frequenza osservata per la mutazione G65A è notevolmente più bassa di quella teorica (1:141) per cui l'elevata prevalenza con cui è stata riscontrata in aree specifiche dell'isola potrebbe essere spiegata con l'esistenza di un "hot-spot" per tale mutazione.

ANALISI DEI VALORI LIPIDICI DEI SOGGETTI CON INFARTO MIOCARDICO ACUTO (IMA) PRESSO L'ASMN-IRCCS DI REGGIO EMILIA NELL'ANNO 2011

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Introduzione. La misurazione dei valori lipidici rappresenta uno dei metodi comunemente utilizzati per identificare individui a rischio di malattia cardiovascolare e per impostare una prevenzione secondaria in soggetti con pregressi eventi cardiovascolari. Nel presente studio sono analizzati i valori lipidici dei soggetti con diagnosi di Infarto Miocardico Acuto (IMA) presso l'Arcispedale S. Maria Nuova di Reggio Emilia nell'anno 2011 allo scopo di valutare le dislipidemie più frequenti in questa popolazione.

Metodi. Il repository informatico provinciale è stato interrogato per la ricerca dei soggetti con diagnosi di IMA nell'anno 2011 (codice diagnosi ICD9-410). Sono state estratte le determinazioni lipidiche (colesterolo totale, LDL, HDL, Lp(a), trigliceridi, Apo-AI, Apo-B) effettuate nel corso dello stesso anno e assegnati ai soggetti i valori lipidici temporalmente più prossimi alla diagnosi.

Risultati. Sono stati identificati 632 soggetti, 181 femmine (28.6%, 74.7 \pm 11.1 aa) e 451 maschi (71.4%, 66.3 \pm 12.3 aa). Le femmine presentano valori di HDL significativamente più elevati e valori di

trigliceridi significativamente più bassi rispetto ai maschi. Analizzando i valori lipidici nei soggetti suddivisi per classi di età si nota che gli individui più giovani (36-69 aa) hanno livelli più elevati di colesterolo totale, LDL, trigliceridi e livelli più bassi di HDL rispetto a quelli in età più avanzata (70-100 aa). Considerando alterazioni lipidiche dei singoli parametri si evidenzia che LDL ≥ 160 mg/dl, trigliceridi ≥ 200 mg/dl e HDL ≤ 35 mg/dl sono presenti rispettivamente nel 9%, nel 12% e nel 34% dei soggetti considerati. HbA1C $\geq 6.5\%$ è stata ritrovata nel 15% dei soggetti considerati.

Conclusioni. I soggetti con IMA in età più giovanile (36-69 aa) presentano un assetto lipidico più sfavorevole rispetto ai soggetti in età più avanzata (70-100 aa). Bassi valori di HDL (≥ 35 mg/dl) rappresentano la dislipidemia singola più frequente (34%). Secondo i criteri del Dutch Lipid Clinic Network, 20 soggetti (3,9%) possono essere considerati possibili/probabili ipercolesterolemici familiari (FH), con indicazione alla misurazione dei valori lipidici nelle famiglie.

EVALUATION OF HDL FUNCTIONALITY IN PEDIATRIC PATIENTS WITH CHOLESTERYL ESTER STORAGE (CESD) DISEASE

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The capacity HDL to act as cholesterol acceptor and to promote cholesterol efflux from cells is the first step of the reverse cholesterol transport (RCT) process and represents an index of HDL functionality in humans. This process can occur by multiple pathways, including aqueous diffusion (AD), scavenger receptor B-I (SR-BI) or ATP-binding transporters A1 (ABCA1) and G1 (ABCG1). Cholesteryl ester storage disease (CESD) is a disorder caused by mutations of LIPA gene encoding for lysosomal acid lipase (LAL). CESD subjects display accelerated and premature atherosclerosis associated with dyslipidemia (high total cholesterol [TC] and LDLc, high triglyceride [TG] and low HDLc). Since evidences from retrospective studies indicate that such lipoprotein derangement already occurs in pediatric age the objective of this work is to evaluate the efficiency of cholesterol efflux capacity (CEC) of HDL from serum of CESD subjects compared to control subjects in pediatric age. Methods: pediatric patients with CESD (n=2) and age-matched control subjects (n=5) were tested for their serum HDL capacity to promote efflux via the multiple pathways. HDL were isolated by (PEG) precipitation. Results: in CESD subjects AD-mediated CEC was lower compared to control subjects (5,82% \pm 0,35 vs 5,15% \pm 0,38; p=0,074), with a trend towards statistical significance; SR-BI- and ABCG1-mediated CEC were significantly reduced in CESD compared to control subjects (-60% and -40%, respectively; p<0,05); on the contrary, ABCA1-mediated CEC appeared to be significantly increased in CESD subjects (+28%; p<0,05). Conclusion: CESD pediatric patients displayed an impaired HDL serum CEC through SR-BI and ABCG1 pathways, that could explain the accelerated atherosclerosis in CESD; on the other hand, the ABCA1-mediated CEC resulted increased in such patients, presumably related to a formation of specific nascent HDL particles able to promote ABCA1-CEC. This can be seen as a sort of compensatory mechanism aimed to counteract foam cell formation and atherosclerosis in these patients.

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 DEFICIENT MICE ARE PROTECTED FROM NEOINTIMA FORMATION IN CAROTID ARTERY INJURY MODEL

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Proprotein convertase subtilisin kexin type 9 (PCSK9) is an important regulator of hepatic low-density lipoprotein (LDL)-cholesterol levels. We have previously shown that PCSK9 is expressed in cultured smooth muscle cells (SMCs) and it is detectable in human carotid atherosclerotic plaques. The aim of the present study was to compare the vascular changes induced by periaortic placement of a non-occlusive constrictive silicone collar for 9 weeks around the common carotid artery of WT and PCSK9 deficient mice. As expected, the plasma cholesterol were mainly in the HDL particles and the PCSK0 KO mice had a significant lower total cholesterol levels (47.1 \pm 8.8 mg/dl vs 13.6 \pm 3.3 mg/dl *P=0.01). Collared carotids of the PCSK9-/- mice (n=6 per group) showed a less marked intimal thickening compared WT mice (19055 \pm 10158 μ m² vs. 34989 \pm 12823 μ m²; *P=0.05), a decreased intimal media ratio (2.54 \pm 1.67 vs. 0.82 \pm 0.70 *P=0.05) and higher lumen area (11821 \pm 7980 μ m² vs. 18830 \pm 8816 μ m²). Carotid lesions of WT mice had an elevated content of SMCs (21.0 \pm 7.56% vs. 10.7 \pm 1.97% P=0.05) and collagen (18.38 \pm 7.90% vs 10.45 \pm 9.11%; **P=0.01) and no difference in macrophage content was detected between the two groups. Cultured SMCs isolated from WT mice showed lower levels of the contractile markers smooth muscle α -actin (-94 \pm 6%; ***P=0.001) and calponin (-74 \pm 18%; ***P=0.001), and increased Col1a1 mRNA levels (2.30 \pm 0.3 fold ***P=0.001) after stimulation with PDGF-BB. Finally, the proliferation rate of PCSK9-/- SMCs was significantly lower compared to PCSK9-/- SMCs reconstituted with PCSK9 encoding plasmid (doubling time 41.2 \pm 1.9 h vs 32.2 \pm 3.1 h; ***P=0.01). Taken together, the present results suggest a favorable action of PCSK9 on neointima formation in response to perivascular carotid, probably facilitating the phenotypic switch of medial SMCs and their proliferation. However, the influence of the different plasma cholesterol profile cannot be excluded. Thus, the direct role of PCSK9 on neointima formation will be investigated, in the future, by generating SMC specific PCSK9 KO mice. The present work was supported by the grant 2012-0549 from Fondazione Cariplo, Italy.

SREBF-1C POLYMORPHISM AFFECTS POSTPRANDIAL LIPID METABOLISM IN NAFLD SUBJECTS

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Background. The prospective data on factors predisposing to NAFLD and associated cardio-metabolic disorders link metabolic syndrome, insulin resistance/hyperinsulinemia and weight gain

to NAFLD; however, not every insulin resistant or obese subject develops NAFLD and NASH, suggesting additional genetic or environmental factors promote liver disease in insulin resistant subjects. A genetic predisposition to NAFLD/NASH is indisputably present. The transcription factor sterol regulatory element-binding protein modulates lipogenesis and insulin sensitivity and has been experimentally connected to NAFLD.

Objective. In population-based studies, SNPs in SREBF-1 gene have been connected to obesity, insulin resistance and T2DM. We aimed at assessing the impact of a common SREBF-1c polymorphism on postprandial lipoprotein metabolism.

Methods. We followed-up 212 nonobese nondiabetic, insulin sensitive participants without NAFLD or metabolic syndrome at baseline, characterized for the common SREBF-1c gene rs11868035 A/G polymorphism, dietary habits, physical activity, adipokine profile, C-reactive protein (CRP), and circulating markers of endothelial dysfunction. A comparable cohort of NAFLD patients underwent liver biopsy, and an oral fat tolerance test with measurement of plasma lipoproteins, adipokines, cytokeratin-18 fragments.

Results. NAFLD patients had a higher postprandial lipemia as compared with healthy controls. In both NAFLD and controls, SREBF-1c GA/AA carriers showed higher IAUC Tg and FFA than GG genotype. SREBF-1c independently predicted postprandial IAUCs of Tg and intestinal and hepatic VLDL1. SREBF-1c GA/AA carriers displayed also a significant increase in IAUC oxLDL and a fall in HDL-C and apoA1 levels.

Conclusions. SREBF1c predisposes to NASH and cardio-metabolic disorders by affecting dietary fat tolerance. SREBF-1c may promote hepatic synthesis of lipotoxic FFA, which may directly promote hepatocyte apoptosis and necroinflammation. In the absence of detectable differences in nutrient intake and physical activity, we may speculate that in at-risk genotypes SREBF-1c-mediated de novo lipogenesis interacts with age-related decline in basal metabolic rate to promote adipose tissue expansion and weight gain.

MONOCYTE INTERACTION WITH THE ENDOTHELIUM: EFFECTS OF CIGARETTE SMOKE

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Circulating monocytes participate in the atherogenic process by adhering to the endothelium and migrating into the intima where they differentiate to macrophages and contribute to plaque growth. Cigarette smoke is a risk factor for atherosclerosis, but it is not clear how it affects monocyte behavior in atherogenesis. We studied the effects of cigarette smoke condensate (CSC) on human monocytes (HM) chemotaxis and transmigration through an endothelial cell (EC) monolayer. Experiments conducted with the Boyden chamber showed that pre-treatment with CSC (7.5-30 µg/ml) for 24 h caused a concentration dependent decrease in HM chemotaxis and transmigration (-55% and -18% vs Control, p 0.05, respectively), paralleled by a reduced expression of the small signaling G proteins Rac 1 GTPase. On the contrary, direct exposure of both HM and EC to CSC increased (+23% vs control, p 0.05) the transmigration of HM, paralleled by a strong stimulation of VCAM1 and ICAM1 expression on ECs, and by a slight increase in monocyte integrin expression. An even more evident enhancement of monocyte transmigration was obtained after the

exposure of both HM and EC to medium conditioned by HM previously incubated with CSC (+265% vs control, p 0.001). Interestingly, incubation with neutralizing antibodies against both MCP1 or IL8 completely abolished the CSC-conditioned medium induced HM transmigration. Finally, treatment with CSC increased the expression of IL8, IL1β, MCP1 and TNFα by HM, and was ablated by pretreatment with PDTC, a well-known NFκB inhibitor. These results indicate that CSC induces HM to release chemotactic factor(s), which may amplify the recruitment and transmigration of inflammatory cells through an EC monolayer; in addition, long-term exposure to CSC reduces HM migratory capacity. Therefore, exposure to CSC affects monocyte behavior and interaction with the endothelium, thus potentially facilitating and/or further aggravating the atherogenic process.

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INFLAMMATION IMPAIRS ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVATION BY HDL IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Aims. The aim of the present study was to evaluate the high-density lipoprotein (HDL) structure and endothelial NO synthase (eNOS) activation capacity in ST-elevation myocardial infarction (STEMI) patients with different acute-phase inflammatory response (APR).

Methods and Results. Forty-five STEMI patients were stratified in quartiles according to the delta CRP level, calculated by subtracting the CRP value at admission from the CRP peak value (APR peak). The HDL structure and HDL capacity to stimulate NO production were evaluated at admission and at APR peak. STEMI patients with a low APR had a completely preserved HDL structure and HDL ability to activate eNOS and promote NO production, which did not change during STEMI. On the contrary, HDL from STEMI patients developing a significant APR had compromised ability to stimulate eNOS and promote NO production, and underwent a significant particle remodelling during STEMI. The defective capacity to stimulate NO production of HDL isolated from STEMI patients with high APR was explained, at least in part, by the reduced PON-1 and S1P content. The HDL ability to promote cell cholesterol efflux through different pathways was preserved in ACS patients independently of the inflammatory response.

Conclusions. The present results extend previous studies reporting an impaired eNOS-activating capacity of HDL from ACS patients, showing that only a subset of patients undergoing STEMI, and in particular those developing an important inflammatory response, have circulating HDL defective in stimulating endothelial eNOS and NO production.

INFLAMMATORY MARKERS AND METALLOPROTEINASES PROFILES PREDICT DEATH IN THE ACUTE PHASE OF ISCHEMIC STROKE TREATED WITH TISSUE PLASMINOGEN ACTIVATOR THROMBOLYSIS

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Background. Inflammatory mediators and metalloproteinases (MMPs) are altered in the acute phase of ischemic stroke and play a detrimental role on severity and hemorrhagic transformation of ischemic brain lesions after thrombolysis. This study aimed to evaluate the effect of inflammatory and MMPs profiles on mortality in stroke patients submitted to thrombolysis.

Methods. Blood was taken at baseline and 24 hours after thrombolysis from 327 patients (mean age 68, mean NIHSS 11.9) with acute ischemic stroke. Circulating molecules were measured using Bio-plex suspension array system [MMP-1, MMP-8, MMP-9, tissue inhibitor of metalloproteinase -1 (TIMP-1), C-reactive protein (CRP), haptoglobin, alpha2-macroglobulin (A2M), interleukin-1 receptor antagonist (IL-1RA), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), tumor necrosis factor-alpha (TNF-alpha), vascular endothelial growth factor (VEGF)]. Baseline values and delta median values [(post tPA-baseline)/baseline] of each parameters were analyzed in 3 month-survivors and non-survivors.

Results. Baseline levels of CRP, haptoglobin, A2M, IL-10, IL-12 and IL-6 and delta values of MMPs 1, 8, 9 and TIMP-1 were significantly different in patients who died with respect to survivors [CRP: 8.25 (2.17-15.49) mg/L vs 2.96 (1.44-8.35) mg/L; haptoglobin: 3.12 (1.24-10.70) mg/mL; A2M: 2.26 (1.83-4.23) mg/mL vs 1.78 (1.24-2.60) mg/mL; IL-6: 6.25 (4.16-11.53) pg/mL vs 4.01 (2.16-7.90) pg/mL; p<0.01 respectively, IL-10: 3.58 (1.16-16.70) pg/mL vs 9.80 (2.99-23.10) pg/mL; IL-12: 18.20 (9.23-42.10) pg/mL vs 24.20 (11.70-52.90) pg/mL, p<0.05 respectively], whereas IL-1RA, TNF-alpha and VEGF levels [IL-1RA: 16.0 (4.98-65.40) pg/ml vs 17.44 (10.3-29.80) pg/ml; TNF-alpha: 1.61 (0.17-3.00) pg/ml vs 2.40 (0.58-5.67) pg/ml; VEGF: 82.2 (43.70-130.5) pg/ml vs 105.8 (56.40-203.7) pg/ml] did not differ between non-survivors and survivors. Adjusting for age, sex, glycemia, baseline NIHSS, history of atrial fibrillation, or congestive heart failure, history of inflammatory diseases or infections occurred within the last 7 days before stroke onset, only delta MMP-9 and baseline A2M remained significantly and independently associated with 3 month-death [OR (95% CI): baseline A2M: 1.49 (1.12-2.00); delta MMP9:1.58 (1.11-2.26), p<0.01]. ROC analysis demonstrated that the addition of baseline A2M and delta MMP-9 (model 2) to a model that included factors known to affect the outcome (model 1) significantly improved the area under the curve for the detection of mortality in ischemic stroke patients [model 1:AUC=0.82 (95% CI 0.75-0.90); model 2:AUC=0.88 (95% CI 0.83-0.93), p<0.05].

Conclusion. Our findings suggest that A2M and deltaMMP-9 are significant and independent markers of mortality and that may be used to improve prediction of unfavourable outcome in the clinical setting of ischaemic stroke patients treated with thrombolytic therapy.

STATINE E RISCHIO DI DIABETE: EVIDENZE DA UN AMPIO STUDIO DI COORTE

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Background. Sono stati riportati risultati contrastanti sull'entità dell'associazione tra l'uso di statine e il rischio di sviluppare diabete. Si è voluto indagare il rapporto tra aderenza alla terapia con statine e rischio di diabete incidente.

Metodi. La coorte dei 115.709 residenti nella regione Lombardia trattati per la prima volta con statine nel periodo 2003-2004 è stata seguita dalla prima prescrizione fino al 2010, identificando i pazienti che iniziavano la terapia con un farmaco antidiabetico o che venivano ricoverati con diagnosi principale di diabete di tipo 2 (esito). L'aderenza alle statine è stata misurata come percentuale di giorni di follow-up coperti dal farmaco prescritto (proportion of days covered, PDC). È stato utilizzato un modello a rischi proporzionali per stimare gli hazard ratio (HR) per l'associazione tra le categorie di PDC e il tempo di insorgenza del diabete. Sono state effettuate alcune analisi di sensibilità per valutare fonti di incertezza sistematica.

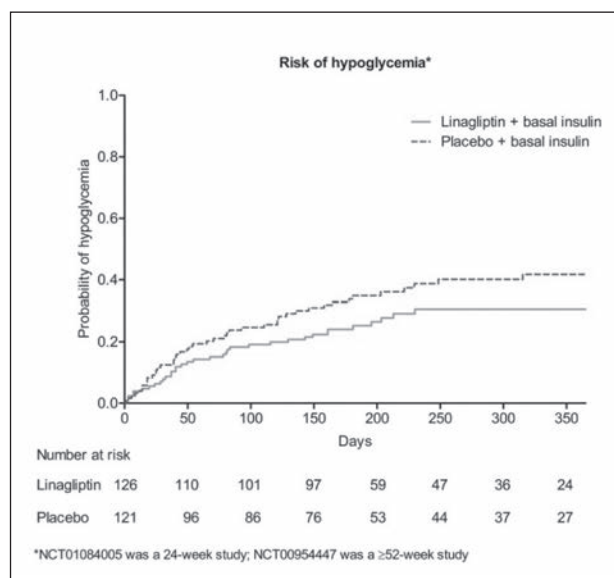
Risultati. Durante il follow-up, 11.154 soggetti hanno sperimentato l'esito. Il 57,3% dei pazienti inclusi mostrava un'aderenza molto bassa o bassa alla terapia statine. Rispetto ai pazienti con aderenza molto bassa (PDC <25%), quelli con bassa (26%-50%), intermedia (51%-75%) e alta (>=75%) aderenza avevano HR (IC 95%) di 1,12 (1,06-1,18), 1,22 (1,14-1,27) e 1,32 (1,26-1,39), rispettivamente. La relazione non cambiava sostanzialmente variando la categorizzazione dell'aderenza o considerando un criterio più restrittivo per la diagnosi di diabete. Nell'analisi di sensibilità, l'associazione tra aderenza al trattamento con statine e rischio di diabete di nuova insorgenza veniva annullata dalla correzione per un fattore di confondimento non misurato di grande importanza per lo sviluppo del diabete, come l'obesità.

Conclusioni. Nella pratica clinica quotidiana, il rischio di nuova insorgenza di diabete aumenta all'aumentare dell'aderenza alla terapia con statine. Tale evidenza deve essere presa in considerazione dai medici, possibilmente monitorando la glicemia nei pazienti in trattamento con statine.

LOWER RISK OF HYPOGLYCEMIA IN ELDERLY TYPE 2 DIABETES PATIENTS WHEN LINAGLIPTIN IS ADDED TO BASAL INSULIN: AN EXPLORATORY ANALYSIS

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Elderly T2DM patients (patients) with long-standing disease often require insulin (INS) therapy, yet hypoglycemia is a major concern. It has recently been shown that linagliptin (LINA) added to stable basal INS in elderly T2DM patients reduced HbA1c by -0.77% vs placebo (PBO), notably with less hypoglycemia. Here we further explore hypoglycemia risk in these patients (n=247; mean ± SD age 74±4 years, HbA1c 8.2±0.8%) on basal INS (baseline [BL] dose 36±25 U/day) from two Phase 3 studies of 24 and ≥52 weeks. Odds ratios (OR) for overall and confirmed hypoglycemia (blood glucose ≤70 mg/dL) were assessed (INS doses did not change notably). Overall (-37%) and confirmed (-34%) hypoglycemia risk was lower with LINA vs. PBO (OR 0.63 [95% CI 0.37-1.10] [Figure] and 0.66 [0.36-1.21], respectively). Significantly less (-59%) confirmed hypoglycemia was found in LINA patients with mild-moderate BL hyperglycemia (HbA1c 7.5-9.0%; OR 0.41 [0.21-0.84]; p=0.014). Similar directional trend in hypoglycemia risk with LINA vs PBO was also observed in patients with BL HbA1c <7.5% (overall OR 0.77) and subgroups for glargine, detemir, or NPH (overall OR 0.74, 0.59, 0.49, respectively). Despite significantly reduced HbA1c and no relevant on-trial INS dose reductions, adding LINA to basal INS appears to decrease hypoglycemia risk. This trend is in stark contrast to other oral agents when combined with INS. The biologic underpinnings of this phenomenon are unclear but deserving further study.



CARDIOVASCULAR (CV) SAFETY OF LINAGLIPTIN IN PATIENTS WITH TYPE 2 DIABETES (T2D): A POOLED COMPREHENSIVE ANALYSIS OF PROSPECTIVELY ADJUDICATED CARDIOVASCULAR (CV) EVENTS IN PHASE 3 STUDIES

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Incidence of CV events is increased in T2D, but the potential for CV risk modulation with glucose lowering therapies is debated. We compared the incidence of CV events and CV mortality in patients with T2D treated with linagliptin (LINA), a once daily DPP-4 inhibitor, with non-lina comparators (comp) in 19 double-blind RCTs (duration ≥12 weeks). CV events were prospectively adjudicated by a blinded independent expert committee.

The primary endpoint was a composite of CV death, non-fatal stroke, non-fatal myocardial infarction, and hospitalization for unstable angina pectoris. Other secondary and tertiary CV endpoints were also assessed. Of 9459 patients, 5847 received LINA (5 mg: 5687; 10 mg: 160) and 3612 comp (placebo: 2675; glimepiride: 775; voglibose: 162). The cumulative exposure (person-years) was 4421.3 for LINA and 3254.7 for comp. In total, 60 primary events were reported in the LINA group and 62 in the comp group (36 in the placebo and 26 in the active comp group). Incidence rates of the primary endpoint (/1000 years at risk) were lower for LINA (13.4) than for the comp group (18.9) as was the hazard ratio (0.78) (Table).

This pooled analysis of adjudicated CV events in a large Phase 3 program continues to support that LINA is not associated with an increased risk for CV events. Potential CV benefits with LINA will be tested prospectively in CAROLINA (NCT01243424) and CAR-MELINA.

| | Linagliptin (n=5847) | Comparator (n=3612) |
|--|----------------------|---------------------|
| Characteristics of study cohort and exposure, according to study arms | | |
| Mean age (years) | 58 ± 11 | 59 ± 10 |
| Female gender (%) | 45.6 | 43.5 |
| Mean baseline HbA1c (%) | 8.1 ± 0.9 | 8.1 ± 0.9 |
| T2D duration >5 years (%) | 54.9 | 56.8 |
| Baseline BMI (kg/m ²) | 29 ± 5.2 | 29.5 ± 5.2 |
| Mean (maximum) exposure (days) | 276 (776) | 329 (804) |

| Impact on primary, secondary, and tertiary CV endpoints, according to study arms | | | |
|---|----------------------------|------|--------------------|
| | Incidence rate/1000 pt-yrs | | HR*(95% CI) |
| Primary CV endpoint | 13.4 | 18.9 | 0.78 (0.55, 1.12) |
| Secondary CV endpoints | | | |
| CV death, stroke, or MI | 9.3 | 14.0 | 0.74 (0.49, 1.13) |
| All adjudicated CV events | 21.5 | 29.1 | 0.82 (0.61, 1.09) |
| Tertiary CV endpoints | | | |
| CV death | 2.4 | 2.4 | 1.04 (0.42, 2.60) |
| Non-fatal MI | 5.1 | 6.1 | 0.86 (0.47, 1.56) |
| Non-fatal stroke | 2.0 | 5.8 | 0.34 (0.15, 0.75)† |
| Transient ischemic attack | 0.2 | 2.4 | 0.09 (0.01, 0.75)† |
| Hospitalization for UAP | 4.9 | 4.8 | 1.08 (0.56, 2.06) |

*Cox proportional model; †Significant lower hazard ratio (upper 95% CI <1.0). BMI, body mass index; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; MI, myocardial infarction; T2D, type II diabetes; UAP unstable angina pectoris

REMNANT CHOLESTEROL CONCENTRATION AND CAROTID INTIMA-MEDIA THICKNESS IN POST-MENOPAUSAL WOMEN

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Background. Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, composed of very low-density lipoproteins and intermediate-density lipoproteins in the fasting state. Cholesterol content of remnants (in the same way as LDL-cholesterol) could be causal for atherosclerosis development, by accumulation in the arterial wall.

Carotid IMT is a well established marker of subclinical atherosclerosis. We tested the hypothesis that fasting remnant cholesterol concentration is associated with subclinical atherosclerosis, marked by carotid thickness of the intima media complex.

Patients and Methods. Three hundred and ninety post-menopausal 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), were offered an ultrasound examination of the carotid arteries and 370 accepted. Blood pressure, serum cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting glucose, were measured in all participants. The thickest IMT of the common carotid wall was selected as ultrasound marker of atherosclerotic disease severity.

Results. Remnant cholesterol concentration was associated with maximum thickness of the carotid arteries ($R=0.17$; $p=0.001$); this association was still significant after adjustment for main cardiovascular risk factors: age, systolic blood pressure, smoking, glucose ($p=0.015$). Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins, which are associated with overweight and obesity. Therefore, we added as covariate BMI in a general linear model that already included main cardiovascular risk factors, but the statistical significance was still retained ($p=0.039$).

Conclusion. In this study we demonstrated an association between remnant cholesterol concentration and extracranial carotid maximum thickness of the arterial wall. This association was independent of the main cardiovascular risk factors. Lowering remnant cholesterol concentration could represent an additional target of therapy in atherosclerotic diseases.

INCREASED C-REACTIVE-PROTEIN AND LACK OF SUBCLINICAL ATHEROSCLEROSIS IN CHILDREN WITH SLEEP-DISORDERED BREATHING

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Background. Several large-scale, prospective, epidemiologic studies have shown that plasma levels of High-sensitivity C-Reactive Protein (Hs-CRP) in adults are an independent predictor of risk for cardiovascular morbidity. Moreover, CRP levels are associated with overweight and obesity. Obesity is a risk factor for sleep-disordered breathing (SDB) in children. Increased carotid intima-media thickness (IMT) is a well recognized marker of subclinical atherosclerosis and is associated with several cardiovascular risk factors both in adults and in children. Sleep-disordered breathing (SDB) in children is characterized by prolonged partial upper airway obstruction and/or intermittent complete obstruction that disrupt normal ventilation during sleep. We evaluated the effect of SDB on Hs-CRP levels and IMT in lean and obese children not selected for snoring.

Patients and Methods. 150 children without other co-morbidities (age 5-15 yrs) evaluated for overweight and obesity in a specialist setting care 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.

In all children IMT was measured with quantitative B-mode ultrasound scans. Children underwent a standard multichannel polysomnography. The apnea-hypopnea index (AHI) was measured overnight: AHI <1 defined controls, AHI ≥1-5=mild SDB, and AHI ≥5=obstructive sleep apnea (OSA).

The sample size of the study was calculated using the following assumptions: 0.055 mm as a relevant difference in carotid IMT between the groups; Standard Deviation of 0.06 mm; alpha error (2-sided): 0.05; beta error: 0.20. Using these criteria a minimum of 19 children per group was necessary to test the hypothesis of a difference in carotid IMT among children with OSA, SDB and controls.

Results. AHI was significantly associated with Hs-CRP concentration ($r=0.32$, $p=0.002$) in all 101 children irrespective of age and sex. Body mass index (BMI) was higher in OSA children versus controls (25.5±7.0 vs 22.1±6.9, $p=0.05$). Obese children had 3.3 times more probability of having OSA (HR 3.3, 95% CI 1.2-9.3, $p=0.02$) than lean children.

Hs-CRP values were significantly higher in children with OSA than in children without ($p=0.011$), but not when BMIz-score was added as covariate. IMT was not associated with AHI or SDB.

Conclusions. The results of this study suggest an association between OSA and Hs-CRP concentrations (mainly mediated by overweight and obesity), reaffirm the role of overweight and obesity in promoting disturbances of sleep-breathing and show, for the first time, the lack of association between SDB and carotid athero-

sclerosis in children. There is scope for prevention in childhood before OSA syndrome causes the irreversible damage to arteries observed in adult patients.

CAROTID CROSS-SECTIONAL AREA IMT AND CARDIAC LEFT VENTRICULAR MASS IN OVERWEIGHT AND OBESE CHILDREN

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Introduction. Carotid intima-media thickness (IMT) is a valid and reliable tool for refining cardiovascular risk to target individuals who need early intervention. Childhood adiposity is associated with adult IMT. We have previously demonstrated that obese children have significantly increased IMT compared with healthy controls. Adult obesity is associated with increased left ventricular mass (LVM), which is an important predictor of adverse cardiovascular outcome.

Patients and Methods. 250 consecutive children (lean or overweight) from 5 to 15 years recruited from a subset of a community sample scheduled for a standard routine visit by their ambulatory pediatrician and 250 consecutive children in the same age range evaluated for obesity in a specialistic setting care, were invited to participate in a study to investigate their cardiovascular markers of atherosclerosis. Overweight and obesity were defined using age- and sex-specific BMI thresholds proposed by the International Obesity Taskforce. 149 out of 250 children (92 lean, 57 overweight) scheduled for routine visit and 217 out of 250 evaluated for obesity accepted to participate to the study. Carotid ultrasound B-mode imaging with IMT measurements was performed in all children. Arterial geometry was further characterized by calculation of carotid cross-sectional area (CSA-IMT) which is a good indicator of arterial wall mass, closely related to cardiac LVM. A complete echocardiographic examination was performed in 77 lean or overweight and 118 obese children: LVM was determined in these children and indexed to height (meters) to the power of 2.7 (LVM/h).

Statistical analysis. The sample size of the study was calculated using the following assumptions: 0.06 mm as a relevant difference in carotid IMT between the groups, standard deviation of 0.08 mm; alpha error (2-sided): 0.05; beta error: 0.20; using these criteria a minimum of 42 children per group was necessary to test the hypothesis of a difference in carotid IMT among children with normal weight, overweight and obesity. The same formula applied to echocardiographic parameters yielded a minimum of 21 children per group, considering 8.5 g/m^{2.7} a relevant difference in LVM/h and 9 g/m^{2.7} as standard deviation. All P values were 2-tailed, with statistical significance set at <0.05. We developed a General Linear Model statistic approach (SPSS, version 17.0) and performed an analysis of covariance with carotid IMT, CSA-IMT and LVM/h as dependent variables; categorized lean/overweight/obese children as fixed factor; age and gender as covariates.

Results. Carotid CSA-IMT was significantly higher in the group of obese children: 9.12±0.10 mm² (mean ±SEM) compared with overweight 8.07±0.20 mm² (p <0.001) and lean children 7.36±0.16 mm²

(p<0.001), the difference between overweight and lean children was statistically significant (p<0.018). Carotid IMT was increased in obese children vs other groups (0.52±0.005 mm in obese, 0.48±0.010 mm in overweight and 0.45±0.008 mm in lean children, p<0.001 both in the group of obese vs overweight and obese vs lean children). Cardiac LVM/h was higher in the group of 118 obese children who underwent cardiac echocardiography (44.6±0.76 g/m^{2.7}) as compared to 24 overweight (34.1±1.68 g/m^{2.7}) and 53 lean children (27.9±1.13 g/m^{2.7}); all p <0.001. P for trend was <0.001 for both vascular and cardiac measurements.

Discussion. Atherosclerosis begins in childhood and results in changes in the structure and function of the arterial tree. Most studies have focused on very obese subjects and less information is available on the more prevalent “healthy” overweight children. The present study demonstrates, for the first time to our knowledge, a higher carotid IMT, CSA-IMT and cardiac LVM/h both in overweight and obese children, with a progressive increase from lean to overweight and obese children. These findings could change the evaluation and treatment of overweight and obesity in youth by focusing on target-organ damage.

HEPATIC STEATOSIS AND CAROTID CROSS-SECTIONAL-AREA OF THE INTIMA-MEDIA-COMPLEX IN HEALTHY-WEIGHT/OVERWEIGHT CHILDREN

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Background. A spectrum of clinicopathologic conditions characterized by excessive accumulation of fat in the liver parenchyma of patients who have no history of alcohol abuse is known to as non-alcoholic fatty liver disease. In children usually we observe only steatosis, which is apparently a benign disease and is associated with obesity, insulin resistance and dyslipidemia. In adults, several studies have demonstrated that hepatic steatosis is a significant risk factor for cardiovascular disease. Carotid intima-media thickness (IMT) is a well recognized marker of subclinical atherosclerosis, and carotid artery cross-sectional area of the intima media complex (CSA-IMC) is a more precise marker, strictly associated with left ventricular hypertrophy. Few studies have shown an association between fatty liver disease and carotid IMT in obese children and adolescents. Aim of the present study was to investigate an association between hepatic steatosis and thickness of the carotid wall in non-obese children.

Patients and Methods. 80 non-obese children without other comorbidities (age 5-15 yrs) scheduled for a standard routine visit by their ambulatory paediatrician accepted to participate in this study. According to International Obesity Task Force criteria, 52 children had healthy weight and 28 children were overweight. In all children IMT was measured with quantitative B-mode ultrasound scans. Arterial geometry was further characterized by calculation of carotid CSA-IMC = $\{\pi \times [IMT + (Dd/2)]^2\} - \{\pi \times (Dd/2)^2\}$. All children underwent abdominal ultrasonography according to a standardized protocol. Sonographic findings were categorized into the presence or absence of steatosis. Diagnosis of steatosis was based on the presence of brightness with clear-cut sonographic contrast between the liver and the right renal cortex in the midaxil-

lary line, according to international guidelines. Subjects with ultrasound-diagnosed fatty liver and/or persistently elevated alanine aminotransferase (ALT) levels were categorized into the hepatic steatosis group.

Results. Hepatic steatosis was present in 11/80 children. Children with hepatic steatosis had greater thickness of the carotid walls as compared to children without steatosis. Carotid CSA-IMC was $9.0 \pm 1.6 \text{ mm}^2$ (mean \pm SD) in the group of hepatic steatosis and $7.0 \pm 1.2 \text{ mm}^2$ in the children without steatosis ($p < 0.001$); the difference between the two groups was statistically significant even after adjustment for age, gender and BMI ($p = 0.002$). Similarly, carotid IMT was increased in children with hepatic steatosis as compared to controls ($0.51 \pm 0.07 \text{ mm}$ vs $0.42 \pm 0.05 \text{ mm}$, $p < 0.001$).

Conclusions. Differently from other studies focused on studying obese children, in the present study we demonstrated an increased carotid wall thickness (both CSA-IMC and IMT) in normal weight or overweight children with hepatic steatosis, independently of BMI. Our results show that hepatic steatosis is a significant marker of increased artery wall thickness and could promote structural vascular changes of the carotid arteries even in non-obese children.

REDUCTION OF CHOLESTEROL WITH NUTRACEUTICAL: RESULTS OF A DOUBLE BLIND STUDY

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A large body of evidence has demonstrated that LDL-C reduction by statins decrease cardiovascular risk. Statin treatment may also lead to non-lipid effects which may improve vascular protection, including an amelioration of endothelial function. On the other hand, despite a good tolerability demonstrated by several studies, statin treatment may lead to side effects, in particular when higher dosages are used. Alternative hypolipidemic treatments are nutraceuticals which are a food, or part of a food, that provides medical benefits. Due to the low efficacy associated to a high tolerability, patients with mild or moderate risk and/or statin-intolerant subjects are the best target of nutraceuticals. Despite a large clinical use, there is a paucity of controlled clinical studies of efficacy and tolerability of this class of drugs. The purpose of the present study, with a double-blind, parallel group, randomized controlled design was to examine the efficacy, safety, and tolerability of a nutraceutical product in hypercholesterolemic patients with a mild/moderate risk previously intolerant to statins or refusing classical pharmaceutical treatments.

We also analyzed the pulse wave velocity as expression of arterial stiffness and, indirectly, of endothelial function. Patients received daily either a nutraceutical-combined pill (NCP), containing red yeast rice 200 mg (corresponding to monakoline 3 mg) or placebo for six weeks. We observed a reduction of 10,4% and 12,2% of total cholesterol and LDL-cholesterol respectively. No significative variation was observed in the placebo group. Pulse wave velocity significantly decreased only in the NCP (-6,5%). Safety parameters did not change during the study and no patient reported myalgia. The mild hypolipidemic effect of red yeast rice is associated to an improvement of arterial function and high tolerability. Therefore we conclude that patients with low or moderate cardiovascular risk and/or statin-intolerant subjects may benefit of this treatment.

DIAPASON: STUDIO OSSERVAZIONALE PROSPETTICO SULLA DIAGNOSI PRECOCE DEL DIABETE MELLITO DI TIPO 2

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Il diabete mellito di tipo 2 (T2DM) è preceduto da una fase pre-clinica caratterizzata da assenza di sintomi che progredisce in maniera silente; di conseguenza è importante identificare, nella popolazione generale, gli individui a rischio.

Lo stato patologico di cui si manifesta con uno squilibrio del metabolismo del glucosio, come (alterata glicemia a digiuno e ridotta tolleranza al glucosio), il cui accumulo colpisce organi bersaglio come occhi, reni e vasi sanguigni.

Razionale. La misurazione della SIF (Skin Intrinsic Fluorescence) è stata proposta come strumento adatto per la diagnosi del diabete. Questa analisi riflette la presenza di AGE (Advanced Glycation Endproducts) nella pelle, che sono prodotti con l'accumulo di glucosio nel tempo.

Scopo. Lo scopo dello studio in oggetto è quello di valutare l'accuratezza diagnostica per prediabete e T2DM di procedure basate sulla valutazione di HbA1c e SIF rispetto all'OGTT (Oral Glucose Tolerance Test) da 75 g., che rappresenta il gold standard per la diagnosi, in soggetti con rischio intermedio/alto secondo il questionario FINDRISC.

Sarà quindi stimata l'incidenza di diabete a 12 e 24 mesi nei soggetti prediabete e nei soggetti con parametri metabolici normali. Infine sarà valutata la relazione tra SIF e la variazione a 24 mesi, rispetto al basale, di alcuni parametri cardiovascolari.

Materiali e Metodi. Nella prima fase dello studio sarà eseguito uno screening di 5000 soggetti di età ≥ 40 e ≤ 75 anni senza diabete, che afferiscono all'ambulatorio del medico di famiglia, mediante il questionario FINDRISC. Nei soggetti con uno score ≥ 9 , cioè con rischio intermedio alto per diabete sarà eseguito un OGTT, il dosaggio di HbA1c e la SIF. In base ai risultati ottenuti i soggetti saranno suddivisi in tre sottogruppi: pazienti con rischio intermedio/alto ma con parametri metabolici normali, pre-diabetici e diabetici. Tutti i soggetti saranno sottoposti ad ulteriori esami, come il quadro lipidico, la funzionalità renale, l'ECG, la misurazione dello spessore intimale carotideo (IMT) e la funzionalità endoteliale, e saranno quindi seguiti per due anni per valutare la comparsa di diabete.

Conclusioni. Al termine di questo studio sarà possibile valutare l'accuratezza diagnostica dalla SIF rispetto alle altre metodiche per la diagnosi precoce di T2DM, stimare l'incidenza di T2DM in soggetti prediabete e con rischio intermedio/alto secondo FINDRISC ma con parametri metabolici ancora normali, valutando l'associazione tra la deposizione di AGE e la comparsa di diabete.

CYCLOSPORINE A IMPAIRMENT OF IN VIVO REVERSE CHOLESTEROL TRANSPORT: FOCUS ON STEROL FECAL EXCRETION

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Macrophage Reverse Cholesterol Transport (RCT) process, by which cholesterol is removed from the foam cells in the arterial wall and transported by lipoproteins (HDL) to the liver for elimination into the feces, is recognized as protective against atherosclerosis. Abundant data indicate that cardiovascular risk is increased in transplanted patients treated with the immunosuppressant drug CyclosporineA (CsA). We demonstrated that CsA impairs the third step of the RCT process in mice, fecal cholesterol excretion, after a 14 days treatment at 50mg/kg/die, without affecting the first and second step of RCT, macrophage cholesterol efflux and its hepatic uptake. To investigate how CsA carries out this effect we analyzed, in mouse hepatic tissues, gene expression of transporters responsible for sterol excretion into the bile, ATP-Binding Cassette transporters G5 (ABCG5) and G8 (ABCG8), and of cholesterol-7-alpha-hydroxylase (CYP7 α), involved in cholesterol conversion into bile acids. RT-PCR analysis revealed an increase in transporters mRNA expression (2,61 \pm 0,87 vs 4,63 \pm 2,09 for Abcg5 and 5,13 \pm 2,23 vs 7,62 \pm 2,53 for Abcg8; mean \pm sd of fold increase/ α -actin) and a significant reduction in Cyp7 α mRNA expression (5.53 \pm 0.43 vs 0.30 \pm 0.05 mean \pm sd of fold increase/ β -actin) in CsA treated mice. In contrast, ABCG5/G8 western blotting analysis showed similar protein content in the two sample groups. We repeated the analysis in mouse intestinal tissues, where ABCG5/G8 are responsible for the excretion of cholesterol in the intestinal lumen, together with RT-PCR of Niemann-PickC1-Like 1 (NPC1L1) protein, involved in cholesterol intestinal absorption. Both RT-PCR analysis on Abcg5/g8 and Npc1l1, and western blotting of transporters expression showed no significant differences between the control group and CsA treated group. These results indicate that CsA treatment impaired the in vivo process of macrophage reverse cholesterol transport, in particular by affecting its third step, fecal sterol excretion. Our results indicate that this negative effect of CsA occurs possibly through the inhibition of CYP7 α expression.

EFFETTI DI UNA COMBINAZIONE DI UN ESTRATTO DI BERBERIS ARISTATA/SILYBUM MARIANUM SUL PROFILO LIPIDICO E LA SECREZIONE INSULINICA IN PAZIENTI DISLIPIDEMICI

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Scopo. Valutare gli effetti di una combinazione di estratto di Berberis Aristata/Silybum Marianum come agente ipocolesterolemizzante e insulino-sensibilizzante in un campione di pazienti sovrappeso e dislipidemici.

Materiali e Metodi. Sono stati arruolati 102 pazienti caucasici, con una condizione di euglicemia, dislipidemici. Dopo un periodo di 6 mesi di run-in durante il quale è stato seguito un regime dietetico e praticata attività fisica, i pazienti sono stati randomizzati ad assumere placebo o una combinazione di estratto di Berberis aristata/Silybum marianum 588/105 mg, 1 compressa a pranzo e una compressa a cena, per tre mesi. Berberis aristata/Silybum marianum e placebo sono stati, poi, interrotti per 2 mesi e ripresi per ulteriori 3 mesi. Abbiamo valutato i parametri antropometrici, la glicemia a digiuno e il profilo lipidico. Tutti i pazienti sono stati sottoposti ad un test al glucagone al basale, alla randomizzazione e alla fine dello studio.

Risultati. Abbiamo osservato una riduzione del peso corporeo dopo i 6 mesi di run-in. Berberis aristata/Silybum marianum ha ridotto il colesterolo totale (-24%), i trigliceridi (-32%) e il colesterolo LDL (-19%) e ha aumentato il colesterolo HDL (+8%) dopo 3 mesi rispetto alla randomizzazione e rispetto a placebo (p<0,05 per tutti). Quando Berberis aristata/Silybum marianum sono stati interrotti, il profilo lipidico è peggiorato (p<0,05 rispetto a 3 mesi dalla randomizzazione); quando Berberis aristata/Silybum marianum è stata reintrodotta, il profilo lipidico è nuovamente migliorato, sia rispetto al periodo di wash-out che rispetto a placebo. Per quanto riguarda il test al glucagone, c'è stato un maggiore aumento di C-peptide e un minor incremento di glicemia dopo il test con Berberis aristata/Silybum marianum rispetto a placebo, al basale e alla randomizzazione.

Conclusioni. Berberis aristata/Silybum marianum è efficace e sicuro nel migliorare il profilo lipidico e la secrezione insulinica in pazienti dislipidemici euglicemici.

FUNCTIONAL CHARACTERIZATION OF NOVEL AMINO ACID VARIANTS IN APOB IN FAMILIAL HYPOBETALIPOPROTEINEMIA

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Introduction. Familial Hypobetalipoproteinemia (FHBL) is a codominant disorder characterized by reduced levels of LDL and apolipoprotein B (apoB) in plasma. In approximately 50% of FHBL cases is due to mutations in APOB gene resulting in truncated apoBs of various size. Only a few missense mutations have been reported so far as the cause of FHBL. In vitro studies have shown that these mutations induce retention of the mutant apoB in the endoplasmic reticulum and impair the secretion of apoB-containing lipoproteins.

We identified two novel amino acid variants (Thr26-27del and Tyr102Cys) located in the N-terminal 1000 amino acids of mature apoB in two hypocholesterolemic blood donors.

Methods. To investigate the functional effect of these variants we constructed plasmids containing human apoB-48 cDNAs harbouring the novel mutations. Rat hepatoma cells (McA-RH7777) were transiently and stably transfected with wild-type or the mutant forms of human apoB-48. The secretion efficiency of human apoB-48 was determined by immunoblotting with human anti-apoB and the incorporation of apoB into medium lipoproteins. Immunocytochemistry was used to monitor the intracellular localization of the mutant proteins. The post-translational stability and the intracellular degradation pathways of mutant apoB-48 was evaluated.

Results and Conclusions. The mutation Tyr102Cys had no effect on apoB-48 secretion. The mutant apoB-48-Thr26-27del almost entirely abolished the secretion of apoB-48 and apoB-containing lipoproteins in the medium, suggesting that the deletion of two amino acids alters the structure of the beta-barrel (the first 267 amino acids) of N-terminal domain of apoB. This mutant apoB-48 appears to be retained in endoplasmic reticulum. The addition of a proteasome inhibitor partially blocked the decay of cellular apoB-48-Thr26-27del suggesting that a significant proportion of the mutant protein was degraded by the proteasomal pathway. The role of autophagy in the degradation of the mutant apoB was excluded.

S17 X LOSS OF FUNCTION (LOF) MUTATION IN ANGIOPOIETIN LIKE 3 (ANGPTL3) IS ASSOCIATED WITH INCREASED PLASMA LIPOPROTEIN LIPASE ACTIVITY AND CHANGES IN LIPOPROTEIN COMPOSITION

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Background. Loss of function (LOF) mutations in ANGPTL3 cause familial combined hypolipidemia (FHBL2) partly through unknown mechanism in humans.

Materials and Methods. We compared lipolytic activities, lipoprotein composition and other lipid-related enzyme/lipid transfer proteins in carriers of the S17X LOF mutation in ANGPTL3 and in age and gender matched non-carrier controls.

Results. Gel filtration analysis revealed a severely disturbed lipoprotein profile and a reduction in size and triglyceride content of VLDL in homozygotes as compared with heterozygotes and non-carriers. S17X homozygotes had significantly higher LPL activity and mass in post-heparin plasma, whereas heterozygotes showed no difference in these parameters when compared to non-carriers. No changes in hepatic lipase (HL), endothelial lipase, paraoxonase 1, phospholipid transfer protein and cholesterol ester transfer protein activities were associated with the S17X mutation.

Conclusions. These results indicate that, while partial Angptl3 deficiency did not affect the activities of lipolytic enzymes, the complete absence of Angptl3 results in an increased LPL activity and mass. Further studies are necessary to understand the low levels of HDL and LDL in subjects affected by FHBL2 due to ANGPTL3 LOF mutations.

ROSUVASTATIN REDUCES MATRIX METALLOPROTEINASE (MMP)-9 AND PLASMINOGEN ACTIVATOR INHIBITOR (PAI)-1 EXPRESSION VIA A MICRORNA-DEPENDENT PATHWAY

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Background. Rosuvastatin, an high-efficacy statin, has been found to deserve several anti-atherosclerotic activities beside the mere LDL-Cholesterol reduction. We have recently found that treatment with rosuvastatin is associated with changes in microRNAs expression (including miR-301a and miR133a/b up-regulation) and in their target genes in human atherosclerotic plaques, including Matrix Metalloproteinase (MMP)-9 and Plasminogen Activator Inhibitor (PAI)-1. The aim of this study was to evaluate the effects of rosuvastatin on macrophage expression of these key genes in plaque destabilization and to highlight the molecular mechanism.

Material and Methods. Macrophage-oriented human monocytic cell line THP-1 were treated with oxidized LDL in order to reproduce the atherosclerotic plaque environment and underwent treatment with rosuvastatin at low (2 μ M) and high (8 μ M) doses. qPCR was used to estimate microRNA and MMP-9 and PAI-1 RNA levels. Western Blot was used to evaluate protein expression. Gain- and Loss-of-function studies with miR-mimics and miR-inhibitors were used to confirm microRNAs involvement in these effects.

Results. Treatment of THP-1 cells with oxLDL results in the formation of foam cells, as confirmed by staining with red-oil. Treatment with rosuvastatin significantly decreased both MMP-9 and PAI-1 mRNA levels and protein. Treatment with rosuvastatin increased miR-301a expression in THP-1 foam cells, whereas very low miR133a/b levels were detected in this cell line. To confirm the effect of miR-133a/b and miR-301a on target gene expression, cells were transfected with miR-133a/b and miR-301a mimics. Treatment with miR-133a, but not with miR-133b, and miR-301a reduced their putative targets, respectively MMP-9 and PAI-1 expression. Despite not predicted by the algorithms, miR-301a also affected MMP-9 expression. Of interest, treatment with an inhibitor for miR-301a reverted the beneficial role of rosuvastatin on MMP-9 and PAI-1 expression.

Conclusions. These data provide the first in vitro evidence that rosuvastatin down-regulate MMP-9 and PAI-1 expression via up-regulation of miR-301a and point out the key role of miR-301a on the beneficial effects of rosuvastatin on atherosclerosis.

IPOVITAMINOSI D E ATEROSCLEROSI PRECOCE IN UNA POPOLAZIONE DI SOGGETTI DIABETICI E DISLIPIDEMICI

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Scopo dello Studio. Per meglio caratterizzare il ruolo della ipovitaminosi D nel processo aterosclerotico, abbiamo valutato la relazione tra i livelli di 25-idrossivitamina D (25OHvitD), i principali fattori di rischio cardiovascolare e lo spessore mediointimale carotideo (IMT), considerato marker di aterosclerosi precoce, in soggetti diabetici e dislipidemici.

Materiali e Metodi. 136 soggetti con diabete di tipo 2 (79 M and 57 F) e dislipidemia diabetica (TG \geq 150 mg/dL, HDL-C \leq 40 mg/dL and LDL-C $<$ 160 mg/dL) sono stati sottoposti a visita medica e prelievo ematico per la determinazione, oltre ad altri parametri metabolici, di 25OHvitD, LDL ossidate (oxLDL) mediante kit Elisa e small dense LDL (sdLDL) mediante tecnica del Lipoprint (elettroforesi su gel di poliacrilamide). Per quanto riguarda l'IMT abbiamo considerato due variabili: il valore massimo (IMTmax) e il valore medio tra tutte le misurazioni (IMTmean).

Risultati e Conclusioni. In una popolazione di soggetti diabetici e dislipidemici abbiamo osservato un'elevata prevalenza di ipovitaminosi D. Bassi livelli di 25OHvitD sono associati a più elevati valori pressori e a un profilo maggiormente aterogeno, caratterizzato da più elevate concentrazioni di colesterolo totale ($p=0.031$) e oxLDL ($p=0.054$) e più alto IMTmax ($p=0.051$). Le concentrazioni di 25OHvitD risultano inversamente correlate in maniera indipendente alle oxLDL ($p=0.035$), dopo correzione per età, sesso, eventi cardiovascolari, stagione e trattamento ipolipemizzante. I livelli di oxLDL sono predittori indipendenti, insieme ad età, sesso ed eventi cardiovascolari, di aumentato IMT ($p=0.017$ per IMTmean and $p=0.040$ per IMTmax). I livelli di 25OHvitD, dopo correzione per età, sesso, eventi cardiovascolari, stagione e trattamento ipolipemizzante, risultano inversamente correlati con IMTmean, senza tuttavia raggiungere la significatività statistica ($p=0.060$). In un modello in cui abbiamo inserito contemporaneamente 25OHvitD e oxLDL, le oxLDL rimangono predittori indipendenti di IMTmean ($p=0.048$). Questi risultati potrebbero aiutare a meglio comprendere la relazione tra ipovitaminosi D e aterosclerosi.

CLINICAL CHARACTERISTICS AND PLASMA LIPIDS IN SUBJECTS WITH FAMILIAL COMBINED HYPOLIPIDEMIA: A POOLED ANALYSIS

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Background. Angiopoietin-like 3 (ANGPTL3) regulates lipoprotein metabolism by modulating extracellular lipases. Loss-of-function mutations in ANGPTL3 gene cause familial combined hypolipidemia (FHBL2). The mode of inheritance and hepatic and vascular consequences of FHBL2 have not been fully elucidated. To get further insights on these aspects, we re-evaluated the clinical and the biochemical characteristics of all reported cases of FHBL2.

Methods and Results. One hundred fifteen FHBL2 individuals carrying 13 different mutations in the ANGPTL3 gene (14 homozygotes, 8 compound heterozygotes and 93 heterozygotes) and 402 controls were considered. Carriers of 2 mutant alleles had undetectable plasma levels of ANGPTL3 protein whereas heterozygotes showed a reduction ranging from 34% to 88%, according to genotype. Compared to controls, homozygotes as well as heterozygotes showed a significant reduction of all plasma lipoproteins, while no difference in Lp(a) levels was detected between groups. The prevalence of fatty liver was not different in FHBL2 subjects compared to controls. Notably diabetes mellitus and cardiovascular disease were absent among homozygotes.

Conclusions. FHBL2 trait is inherited in a co-dominant manner and the lipid-lowering effect of 2 ANGPTL3 mutant alleles was more than 4 times larger than that of one mutant allele. No changes in Lp(a) were detected in FHBL2. Furthermore, our analysis confirmed that FHBL2 is not associated with adverse clinical sequelae. The possibility that FHBL2 confers lower risk of diabetes and cardiovascular disease warrant more detailed investigations.

ENDOPLASMIC RETICULUM STRESS AND NRF2 REPRESSION IN CIRCULATING CELLS OF TYPE 2 DIABETIC PATIENTS: ROLE OF OXIDATIVE STRESS AND INFLAMMATION

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Inflammation, oxidative and endoplasmic reticulum (ER) stress play a key role in the pathogenesis of type 2 diabetes mellitus (T2DM), contributing to pancreatic β cells loss and insulin resistance. In fact, under chronic high glucose conditions, increased insulin biosynthesis overwhelms the ER folding capacity. So, the unfolded protein response (UPR), that promotes cell adaptation and survival by inducing the expression of protective mechanisms such as the nuclear erythroid related factor 2 (Nrf2)/antioxidant related element (ARE), is insufficient, switching to apoptosis.

In this study we evaluated oxidative stress markers (oxidation product of phospholipid 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine, oxPAPC, and malondialdehyde, MDA), the UPR and ER apoptosis, the activation of the pro-inflammatory nuclear factor (NF)- κ B with its inhibitory protein I κ B α and finally the expression of the protective Nrf2 and heme oxygenase-1 (HO-1) in peripheral blood mononuclear cells (PBMC) of T2DM patients, in order to explore the possible links between oxidative stress, inflammation and ER stress in circulating cells, on the basis of previous data only referred to β cells.

15 T2DM patients without glycaemic target control, with history of disease \geq 10 years but without any other cardiovascular risk factor nor T2DM related organ damage, were matched with 15 healthy controls (C). The concentrations of oxPAPC (in PBMC and plasma) and of MDA (in plasma) were significantly higher in T2DM patients respect to C. The expression of glucose-regulated protein 78 kDa (GRP78/BiP) as representative of UPR, and of C/EBP homologous protein (CHOP) as representative of ER-apoptosis, were significantly higher in T2DM patients. I κ B α expression was significantly lower in T2DM patients as well as Nrf2 and HO-1 expression.

In circulating cells of chronic T2DM patients without glycaemic target achievement there is an activation of UPR and of ER apoptosis, that may be related to the augmented oxidative stress and inflammation, without a corresponding Nrf2/ARE defence activation.

FATTY LIVER AND SERUM FETUIN-A LEVELS IN DIFFERENT ANATOMICAL SITES OF ATHEROSCLEROSIS

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Background. Non-alcoholic fatty liver disease (NAFLD) is deemed to be an independent risk factor for cardiovascular disease (CVD) which is the leading cause of death in these individuals. Although the pathogenic mechanisms linking NAFLD with CVD are

incompletely understood, the role played by fetuin-A has gained increasing interest.

Aims. To compare the prevalence of NAFLD and serum fetuin-A levels in patients with atherosclerosis in 4 different anatomical sites: carotid arteries, lower extremities, abdominal/ thoracic aorta and coronary arteries.

Methods. NAFLD was diagnosed by ultrasound imaging, standard anthropometric indices and metabolic parameters were also recorded, fetuin A was determined in serum by ELISA.

Results. The prevalence of NAFLD was 53.80 %, a percentage higher than that observed in the general population. Contrary to what is observed in the general population, in patients with arterial disease NAFLD is not more prevalent in males. Patients with peripheral arterial disease had the greatest number of factors of metabolic syndrome (MS) (3.50 (2.75-4.00), $P=0.036$) and the lowest HDL cholesterol levels (38.34 \pm 9.93 mg/dl, $P=0.008$); visceral fat thickness was higher in the Coronary artery disease (CAD)+ group (61.75 \pm 23.87 mm, $P=0.000$) than the other groups. Serum concentrations of fetuin-A were significantly different between the four groups ($P=0.000$) with the highest values in the CAD+ group (334.04 \pm 123.91 μ g/ml), in each group the fetuin-A was more elevated in patients with NAFLD than in non-NAFLD.

Conclusions. Data seem to suggest that atherosclerotic disease in different vascular anatomical sites is associated with multiple risk factors. Fetuin-A may be involved in atherosclerosis associated with NAFLD.

A NOVEL PARTIAL DELETION OF LDL RECEPTOR GENE IN AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA (ADH-1)

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Introduction. Autosomal Dominant Hypercholesterolemia (ADH) is a monogenic disorder which predisposes to atherosclerosis and premature coronary heart disease. More than 90% of the ADH cases are due to a variety of mutations in LDL-receptor (LDLR) gene (ADH-1).

Materials and Methods. A healthy 17 years old male was referred to lipid clinic for elevated total and LDL cholesterol after routine medical examination. His serum LDL cholesterol (LDL-C) was 307 mg/dl (HDL-c 33 mg/dl, TG 83 mg/dl, ApoA1 96 mg/dl, ApoB 228 mg/dl and Lp(a) 54 mg/dl). He had neither xanthomas nor xanthelasma; carotid artery intima-media thickness was 0.65 mm, increased for age and sex; treadmill stress test was negative. His mother and his two siblings had hypercholesterolemia but the family history was negative for CHD. The Dutch score was >8 points, suggesting definite ADH.

Results. The sequence of LDLR gene was negative. However the analysis of LDLR gene with MLPA (Multiplex Ligation Dependent Probe Amplification) revealed that the patient was heterozygous for a deletion involving exons 13, 14 and 15. This deletion was confirmed by PCR amplification of intron 12-intron 16 region and the identification of the deletion break-point showed that first 45 bp of exon 13 joined to the partially deleted intron 15, resulting in a

deletion of 4272 bp combined with a duplication of 11 bp of intron 15. The break point was located within an Alu sequence. The same deletion, predicted to induce a disruption of the mRNA processing, was found in proband's hypercholesterolemic relatives. This is the third deletion of exons 13-15 found in Italian ADH patients.

Conclusions. Routine laboratory testing can be useful to identify asymptomatic ADH patients. The search for major rearrangements of LDLR gene by MLPA is highly recommended in patients with definite/probable ADH, negative for mutations detectable by nucleotide sequencing.

ECO-DOPPLER ABNORMALITIES IN RELATION TO LDL CHOLESTEROL IN FAMILIAL HYPERCHOLESTEROLEMIA

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Familial hypercholesterolemia (FH) is an autosomal, codominant disease primarily caused by mutations in the LDL-receptor (LDLR) gene. The aim of this study is to evaluate the impact of LDL-Chol levels on extension of subclinical CVD in FH. Baseline levels of LDL (before starting lipid-lowering therapy) were determined in 66 patients (56,1% women; 42±14 years) with genetic features of FH without diabetes or other metabolic disorders. In our population, we have been identified 22 different mutations in the region that encodes LDLR heterozygous, 6 different mutations in double heterozygous and 1 homozygous. Patients were defined having higher LDL, when their LDL levels were higher than the median value (303 mg/dL) observed in the whole patients sample. All patients underwent standard carotid artery B-mode and Doppler ultrasound. In the whole sample, prevalent CVD was significantly higher in patients with higher LDL-Chol compared to those with lower LDL-Chol (35% vs 12%), independently of age (p 0.031). Patients with higher LDL-Chol had increased IMT, higher number of carotid plaques and more severe carotid stenosis (>50%), independently of age, gender, hypertension and CVD (all p=0.002). Within FH population, elevated LDL-Chol levels are strongly and independently related to carotid atherosclerosis.

(DIS)-AGREEMENT AMONG CARDIOVASCULAR RISK CALCULATORS IN CLINICAL PRACTICE

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Background. Estimation of global cardiovascular risk is often required by guidelines and assists the physician in the management of patients. Moreover, the Italian National Institute of Health (SSN) recommends the use of the estimated cardiovascular risk in order to select patients suitable for full coverage for preventive medications. Anyway, different guidelines apply different algorithms and the research in consistence of these calculators in clinical practice

is still limited. Thus, aim of our study was to assess the consistency of these algorithms and to evaluate their correlation with arterial stiffness, a subclinical and early marker of atherosclerosis.

Methods. After searching on Pubmed, we selected 13 calculators to be evaluated in our study. Using these calculators, we estimated the cardiovascular risk for 500 primary prevention patients evaluated in our clinic between 2011 and 2013. Vicorder device was used to assess Pulse Wave Velocity in our patients.

Results. The overall concordance among the calculator was 44,0±7,0%, and the mean inter-calculators agreement was $k=0,224\pm0,056$ (mean of Cohen k coefficients). Focusing on the calculator mainly used in Italy, we found that the "Progetto Cuore" and SCORE algorithms stratified a significantly higher percentage of patients (62,4% and 76,8%, respectively) into the lowest risk category in comparison with ESH/ESC Guidelines (45,6%), and Framingham (49,0%; p<0.001). Conversely, patients included in the highest risk category were sensibly lower by using "Progetto Cuore" algorithm (1,4%) and SCORE (1,7%) when compared with ESH/ESC (13,3%) and Framingham (12,2%). Finally, we found that Pulse Wave Velocity directly correlates with risk estimated by "Progetto Cuore", Framingham, and ESH/ESC algorithms. A lower grade of correlation was found with EuroSCORE.

Conclusion. Our data show a substantial inconsistency among the different cardiovascular risk algorithms, despite a correlation with arterial stiffness was found. Due to these differences, the choice of cardiovascular risk calculator has a great impact on risk categorization with implications for guidelines recommending therapies based on specific calculators.

PLASMA CREATININE LEVELS, ESTIMATED GLOMERULAR FILTRATION RATE AND CAROTID INTIMA MEDIA THICKNESS IN MIDDLE-AGED WOMEN: A POPULATION BASED COHORT STUDY

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The relationships between high Creatinine (Cr) levels or low estimated Glomerular Filtration Rate (eGFR) and common carotid Intima Media thickness (IMT) have 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 Cr and eGFR were measured in 310 women, as a part of 5.062. In this group carotid ultrasound examination (B-Mode imaging) was performed and mean max IMT was calculated.

Women were classified by Cr levels >1 mg/dL or eGFR <56 ml/min. Women with Cr >1 mg/dL or eGFR less than 56 ml/min did not differ from the rest of the cohort with regard to age, prevalence of diabetes, smoking, obesity or overweight, high blood pressure (Systolic Blood pressure >130 mm Hg, or Diastolic Blood pressure >80 mm Hg), high LDL cholesterol (>100 mg/dL), low HDL cholesterol (<40 mg/dL), high Triglycerides (>150 mg/dL). Women with creatinine >1 mg/dL (corresponding to the 90th percentile of creatinine distribution) or eGFR less than 56 ml/min (corresponding to the 5th percentile of eGFR distribution) had relatively more carotid plaques as compared to the rest of the cohort.

Multivariate logistic analysis, after adjustment for age, demonstrated a significant association between Cr (>1 mg/dL) and IMT (≥1.2 mm): OR 4.12 (C.I 1.22-13.86), p=0.022; or eGFR (<56 ml/min) and IMT (≥1.2 mm): OR 4.31 (C.I 1.27-14.66), p=0.019.

These findings on an independent relationship between Cr and common carotid plaques in this population of middle aged women, independently of age, suggest the value of screening for early carotid disease in asymptomatic middle aged-women with mild renal insufficiency, in order to predict those at relatively higher risk for future cardiovascular events.

LIPIDOMICS OF HUMAN SKIN FIBROBLASTS IN NEIMANN-PICK DISEASE TYPE C

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Neimann-Pick Disease type C is a hereditary lysosomal storage disease due to mutations of the NPC1 or NPC2 genes. The products of these genes encodes for components of a lysosomal machinery that transport different kinds of lipids across the lysosomal membrane. The main abnormalities of NPC1 deficiency phenotype is the accumulation of free cholesterol (FC) and sphingolipids (GL) in brain, liver and other organs cells. Neurodegeneration and hepato-splenomegaly are common findings. The NPC disease, in his severe onset, leads to a precocious exitus, though the inhibition of glyco-SL synthesis by miglustat has shown some clinical benefit. Recently the physiopathological mechanism has been partly elucidated, being the first step represented by the disruption of lysosomal calcium homeostasis due to sphinganine accumulation. NPC1 Human skin fibroblasts (HSF) accumulate FC, as shown by filipin staining. The exact composition of the whole NPC1-HSF lipidome is currently poor characterized. We developed a fully automated method to resolve individual lipids species from cells extracts by LC-ESI-MS in a Thermo Scientific Q exactive LC MS/MS apparatus. This methods allowed to obtain relative quantification and identification of more than five hundreds of different compounds using both positive and negative ionization modes. We used this method to compare the lipidome profiling of free living NPC-HSF with healthy control-HSF. Hopefully the results will add useful information to support the understanding of the NPC pathophysiology.

THE "VERY HIGH" RISK PATIENTS IN A "LOW" CARDIOVASCULAR DISEASE COUNTRY: PREVALENCE, TREATMENTS, TARGETS AND OUTCOME

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Background. Euroaspire III showed that the guidelines targets are hardly reached in clinical practice. We analysed the prevalence, clinical findings, treatments and 1-year outcome of "very high" CV

risk (VHCVR) patients at enrolment in the "Trieste Registry of CV Diseases" (TRCVD).

Methods. From November 1, 2009 to December 31, 2012, the TRCVD enrolled 19589 patients with CV ambulatory evaluation. Clinical data were derived from the E-data chart for outpatient clinic (Cardionet[®]), and collected in a regional Data Warehouse. Patients were stratified for CV risk according to European Guidelines for CV Prevention 2012.

Results. 9279 patients (47.4%) were classified at VHCVR (age 70.7±11.2 years, 58% males) according to the presence of ischemic heart disease (HD) (55%; 44.2% with myocardial infarction), vascular disease (28%), diabetes mellitus associated to organ damage or other risk factors (38.6%), severe chronic kidney disease (CKD) (GFR <30 mL/min/1.73 m²) (4.4%), a calculated SCORE ≥10% (0.9%). ACE and/or AII inhibitors were prescribed in 54.9% of patients, betablockers in 37.4%, statins in 50% (61.9% in ischemic HD), antiplatelets and/or anticoagulants in 70.2%. Target levels of risk factors was overall satisfied in 13.3% for LDL <70 mg%, 49.5% for BP<140/90 mmHg, 64% for Hb1AC <7% in diabetes. 1-year all cause mortality was 3.4% in VHCVR pts (vs 2.5% in others, p=0.001). 1-year mortality or CV hospitalization was 18% in VHCVR pts (vs 8.6% in others, p<0.001). Combined events was around 17-22% in all groups but pts with GFR<30 mL/min/1.73 m² (31.4%).

Conclusion. Among patients with VHCVR, an aggressive and targeted intervention seems to be mandatory to induce positive lifestyle modification, increase drug prescription and dosage, improve the achievement of target levels of risk factors and outcome. Achievement of LDL target and outcome in severe CKD patients seem the more critical and urgent issues to face.

THE EFFECT OF 14 DAYS OF BED REST ON THE LIPID AND INFLAMMATORY PROFILE ON THE ELDERLY

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Introduction. Physical inactivity greatly reduces the quality of life of the elderly, personal autonomy and consequently increases the demand for formal and informal care. The Bed Rest is a good experimental model to study the functional and metabolic changes that occur during physical inactivity. The PANGeA group has used the Bed Rest model, for the first time in adult/elderly, to study the acute effect of the confinement to bed.

Methods. Eight young subjects (Young - age 18-25 years) and sixteen adult/elderly subjects (Elderly - age 55-65 years) with similar BMI, and body composition, were enrolled. Anagraphic and anthropometric data, body composition, blood samples,..... were collected at baseline (BR1) and after bed rest (BR14). In addition, eight of the sixteen elderly subjects during Bed Rest were subjected to a Brain training with specific software (Elderly⁺). We have studied the changes in lipid and inflammatory profile occurred after 14 days of bed rest.

Results. At baseline, the Elderly subjects were different from the Young for the levels of total (204±39 mg/dl vs 151±15 mg/dl; P 0,002) and LDL cholesterol (137±34 mg/dl vs 89±12 mg/dl; P 0,002). In the Elderly total and LDL cholesterol was reduced by 13% and 16% (T- test for paired data, respectively P <0,001 and P 0,002), in the young people was reduced by 4.3% and 4% (n.s.). In the Young HDL cholesterol was reduced by 17% (P 0,065), no change in the Elderly. In the Elderly⁺ HDL cholesterol increased,

although not significantly, by 10%, while in the Elderly was reduced by about 10%. In the Elderly non-HDL cholesterol was significantly reduced by 16% (Elderly reduce 14% and Elderly⁺ 19%), no change in the Young. Total Cholesterol/HDL Cholesterol ratio was reduced by 7% in the Elderly (Elderly [5,16±0,90 to 5,22±1,35] +2%, Elderly⁺ [5,00±1,25 to 4,13±1,5] -18%) while it increased by 15% in the Young (3,57±0,55 to 4,09±0,71), P ANOVA 0,03. No significant changes were observed in serum triglycerides levels.

Elderly and Young weren't significantly different in basal levels of C-Reactive Protein (CRP) and TNF- α . No significant changes of CRP were observed after Bed Rest. TNF- α levels increased significantly in the Young and Elderly.

$\Delta\%$ Total Cholesterol negatively correlated with $\Delta\%$ CRP, while the $\Delta\%$ Triglycerides positively correlated with the $\Delta\%$ Fat Mass. Correlations between $\Delta\%$ of the other lipid components and body composition and inflammatory status weren't observed in the non parametric analysis.

Conclusion. In the Young, Bed Rest induces a significant worsening of the lipid profile, while in the Elderly, especially Elderly⁺, an apparent improvement. Bed Rest increases TNF- α , but not CRP.

METABOLIC SYNDROME PREDICTS CARDIOVASCULAR ISCHEMIC EVENTS IN PATIENTS WITH ATRIAL FIBRILLATION ON TREATMENT WITH ORAL ANTICOAGULANTS

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Objective. Metabolic syndrome (MetS) is a condition associated with an increased risk of cardiac and cerebral ischemic events. A higher risk to develop atrial fibrillation (AF) has been well recognized in patients with MetS. Studies reporting on the prevalence of MetS in AF population are still lacking.

Furthermore the impact of MetS on the incidence of cardiovascular events in patients with AF taking oral anticoagulants (OAT) has never been investigated.

Methods. We prospectively analyzed 810 anticoagulated patients with AF. Exclusion criteria were: prosthetic valves, active cancer, chronic inflammatory diseases. MetS was defined according to modified ATP-III criteria.

Results. Patients were followed-up for a median time of 25.4 months (IQR: 15.1-46.2; 2033 patients/year). Mean age was 73.1 years; 55.3% were male and 58.6% met the criteria for MetS. At baseline patients with and without MetS were significantly different for BMI, waist circumference, CHA₂ DS₂-VASc score, HDL cholesterol, triglycerides, glycaemia, hypertension, diabetes, heart failure, history of myocardial infarction. Use of ACE inhibitors/sartans, beta blockers, oral hypoglycemic agents, insulin and statins was significantly more frequent in patients with MetS. Eighty nine patients (10.98%) experienced a primary outcome during follow-up: 31 MI/Revascularization, 22 stroke/TIA and 36 cardiovascular deaths. Kaplan Meier curves showed that AF-MetS patients had more probability of experiencing MACEs (log-rank test: p=0.003) compared to AF ones without MetS; a sub-analysis demonstrated that AF-MetS patients had more probability of experiencing AMI/Revascularization compared to those without (log-rank test: p=0.005). In a Cox proportional

hazard model, age (≥ 75 years), history of stroke/TIA, history of MI/cardiac revascularization and MetS independently predicted cardiovascular events.

Conclusion. In AF patients receiving OAT, MetS is and independent predictor of cardiovascular events and, in particular, of AMI/Revascularization.

FLOW-MEDIATED DILATION IS IMPAIRED IN DIABETIC PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE: RELATION WITH POOR GLYCEMIC CONTROL

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Objective. Nonalcoholic fatty liver disease (NAFLD) has a high prevalence in the general population. Brachial artery flow-mediated dilatation (FMD) is a surrogated marker of early atherosclerosis. Few data regarding FMD in diabetic patients with NAFLD are present in literature.

Methods. We recruited 200 patients with NAFLD and 64 controls. Diagnosis of NAFLD was made through ultrasound evaluation. FMD of the brachial artery was performed and glycated hemoglobin (HbA1c) and lipid profile was measured in all patients. Patients with chronic viral liver infection or cirrhosis were excluded.

Results. Mean age was 54.1±12.1 years. A significant high BMI (p<0.001), high waist circumference (p<0.001), low HDL-cholesterol (p<0.001), high triglycerides (0.001) and high blood glucose (0.003) was present in patients with NAFLD. FMD was reduced in patients with NAFLD as compared to controls (4.6±2.8 vs 5.5±2.9 p=0.031). In the NAFLD group, diabetic patients showed a further reduction in FMD against non-diabetic patients (3.9±2.6 vs 4.9±2.8 p=0.025). In particular, patients with a value of HbA1c >6.5% had the lowest value of FMD compared to patients with HbA1c <6.5% (3.7±2.6 vs 4.8±2.8 p=0.038).

Conclusion. FMD is reduced in diabetic patients with NAFLD. In particular FMD seems to be correlated to a poor glycemic control, as shown in patients with HbA1c >6.5%. Tight glycemic control is needed in patients with NAFLD to reduce endothelial dysfunction.

VITAMIN B12 AND FOLIC ACID LEVELS IN CHILDREN WITH GENETIC DIAGNOSIS OF HETEROZIGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Aim. to determine vitaminB12 (vB12) and folic acid (fa) levels in children with and without genetic diagnosis of heterozygous familial hypercholesterolemia (heFH) at their first access at our Lipid Clinic. The background of this study is that children with heFH usually have one or both hypercholesterolemic parents, so

they are frequently restricted in dietary quality (lipid intake, milk, meat), without any medical or nutritional control.

Patients and Methods. 137 severely hypercholesterolemic children (median age 8.6 y, 134 male/144 female), with genetic diagnosis of heFH, no ongoing pharmacological treatment, vitamin supplementation or secondary causes of hypercholesterolemia, were evaluated for: anthropometric measures, pubertal stage, twelve-hour fasting blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and Triglycerides (TGC) by enzymatic method, vB12 and fa by electrochemiluminescence. They were divided into two groups according to pubertal stage (97 Tanner =1, 40 Tanner ≥2). These two groups were not different for basal characteristics or lipid profile.

Statistics. Student's t test or Mann-Whitney test for independent samples.

Results. vB12 (pg/ml, mean±ds) and fa (ng/ml, mean±ds) levels were, respectively 761.7±261 and 8.6±3.7, within normal range and with no differences between sexes. vB12 and fa levels in Tanner =1 and Tanner≥2 were, respectively: vB12 813.7±261.7 vs 635.6±216.1, p=0.001, fa 9.3±3.7 vs 6.9±3.1, p=0.001.

Conclusions. vB12 and fa levels were within normal range in children with genetic diagnosis of heFH not on nutritional or pharmacological treatment, with fa at the lower end. Children with ongoing puberty present lower levels of vB12 and fa than prepubertal ones, and this differences is statistically significant. This result might be caused by a longer-lasting restricted and uncontrolled diet in older children and by higher metabolic requests during puberty. These data are worth for further investigations.

LIPOPROTEIN(A) [LP(A)] IN A POPULATION OF HYPERCHOLESTEROLEMIC CHILDREN WITH GENETICAL DIAGNOSIS OF HETEROZIGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HEFH)

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Aim. To evaluate plasma Lp(a) levels in children with and without genetically-confirmed heFH and to determine the association between Lp(a) levels and CVD family history (famH) in severely hypercholesterolemic (shc) children at their 1st access to our Lipid Clinic.

Patients and Methods. 278 shc (median age 8.6y, 134 M/144 F) with positive famH for hypercholesterolemia and/or premature CVD, no ongoing pharmacological treatment or secondary causes of hypercholesterolemia, were evaluated for: anthropometric measures, 12-hour-fasting blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and Triglycerides (TGC) by enzymatic method, Lp(a) levels by nephelometry and genetic analysis for heFH by PCR.

Statistics. Student's t test or Mann-Whitney test for independent samples.

Results. The 278 patients were divided in 2 groups according to genetically-diagnosed heFH. 137 children had a mutation of the LDL-receptor (FH-group), 141 did not (non-FH-group).

Lipid profile (mg/dl, mean±ds) in the FH-group and in the non-FH-group was, respectively: TC 268.4±53 vs 213±46 (p<0.0001), LDL-C 194.4±55 vs 136.3±34. (p<0.0001), HDL-C 56.7±11.8 vs 59.2±13.6 (p=0.157), TGC 78±38.1 vs 74.9±33.7 (p=0.628), Lp(a) 21.3±23.9 vs 29.6±36.8 (p=0.440).

FamH in the FH-group and non-FH-group was, respectively: positive in 71 and 74 children (CVD+) (1st degree relatives in 21 and 11, 2nd in 50 and 63), negative in 62 and 66 (CVD-), unknown in 4 and 1. The lipid profile was comparable in CVD+ and CVD-, p>0.1 There was no statistically significant difference in Lp(a) mean levels in CVD+ vs CVD-.

Conclusions. In our population, there is no difference in Lp(a) levels in heFH and non-heFH children and no correlation with famH for CVD. The possible explanation could be the low median age of shc and the statistical power of offspring study, which is smaller than that of case-control studies.

STATINS DECREASE THROMBIN GENERATION AND FACTOR VIII ACTIVITY IN PATIENTS WITH HYPERLIPIDEMIA

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Aim. Statins therapy is widely used to reduce plasma cholesterol levels and the related cardiovascular risk. The effect of hypercholesterolemia as well as its reduction by statin on the coagulation system is not completely elucidated. Aim of our study was to see whether thrombin generation, as assessed upon triggering plasma coagulation by small amount of tissue factor, a situation mimicking what occurs in vivo, is modified, and to what extent, by treatment with statins in patients with hyperlipidemia.

Methods. Eighty hypercholesterolemic patients (familial hypercholesterolemia, familial mixed hyperlipidemia) and twenty-two healthy subjects were enrolled in this study. Endogenous Thrombin Potential (ETP), factors VIII, protein C activity and lipid concentrations were measured in patients before and after three months of statins therapy.

Results. After statins therapy, median plasma cholesterol level decreased from 325 mg/dL to 211 mg/dL (p<0.001) as well as triglycerides levels (from 116 mg/dL to 115 mg/dL) (p=0.004) whereas plasma HDL cholesterol level increased (from 48 mg/dL to 132 mg/dL) (p<0.001).

Median ETP value, that was significantly higher in patients at pre-treatment as compared to controls (2,372 nM·min vs. 2,086 nM·min) (p<0.05), decreased significantly at post-treatment (2,080 nM·min, P<0.001). Median factor VIII activity decreased significantly after treatment (77% vs. 60%, p<0.01) as well as median protein C activity [126% (84-194) vs. 120% (92-178), p=0.005].

In patients, factor VIII activity correlated with the ETP both at pre-treatment, (rho =0.467, p<0.001) and after treatment (rho =0.440, p<0.001). The median difference of factor VIII activity recorded at pre- vs. post-treatment was significantly correlated with the median difference for the ETP recorded at pre- vs. post-treatment (rho =0.629, p<0.001).

Conclusions. In the present work we demonstrate that statins reduce considerably thrombin generation and FVIII activity in hypercholesterolemic patients. These effects further integrate our knowledge on the beneficial role of cholesterol reduction by statins on the cardiovascular system.

ACUTE EFFECT OF DARK CHOCOLATE ON OXIDATIVE STRESS AND FLOW-MEDIATED DILATION IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

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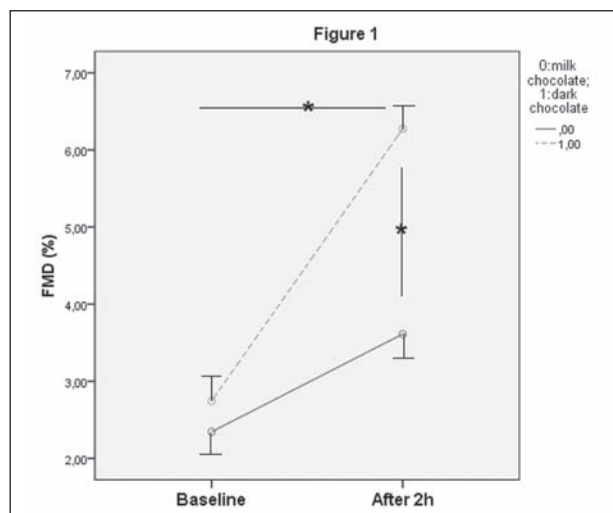
Background. Peripheral arterial disease (PAD) is a clinical setting characterized by an exceptionally high risk for cardiovascular events. Oxidative stress seems to play a role in impairing flow-mediated dilation (FMD) and contributing to atherosclerosis in patients with PAD. Cocoa seems to exert artery dilatation via oxidative stress inhibition.

Objectives. To investigate whether in PAD patients, dark chocolate elicits artery dilatation via down-regulation of NOX2, the catalytic core of NADPH oxidase.

Methods. Flow-mediated dilatation (FMD), oxidative stress (as assessed by urinary isoprostanes excretion), nitric oxide generation (as assessed by serum levels of nitrite/nitrate (NOx)), NOX2 activity (as assessed by blood levels of soluble NOX2 derived peptide (sNOX2-dp)) and serum epicatechin were studied in 18 PAD patients in a crossover, single-blind study. Patients were randomly allocated to 40 g dark chocolate (>85% cocoa) or 40 g of milk chocolate (≤35% cocoa). FMD, urinary isoprostanes, NOx and sNOX2-dp, platelet oxidative stress and NOX2 activation, were assessed at baseline and 2 h after chocolate ingestion.

Results. After dark chocolate intake, urinary isoprostanes and sNOX2-dp significantly decreased and FMD (Figura 1) and NOx significantly increased in PAD patients. No changes of the above variables were observed after milk chocolate intake. Serum epicatechin significantly increased only after dark chocolate ingestion. Ex-vivo study showed, in platelets of PAD patients, that after dark chocolate, 8-iso-PGF₂α and NOX2 activation significantly decreased; no effect of milk chocolate was detected.

Conclusion. This study suggests that in PAD patients, cocoa enhances artery dilatation by lowering of NOX2 activation. These results could open new therapeutic strategies to counteract oxidative stress and atherosclerotic progression in PAD.



ASSOCIAZIONE TRA EPATOPATIA STEATOSICA NON ALCOLICA E SCLEROSI VALVOLARE AORTICA IN PAZIENTI CON DIABETE TIPO 2

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Premessa e Scopo dello Studio. La sclerosi valvolare aortica (AVS) è un importante predittore di aumentata mortalità cardiovascolare. Recenti dati hanno documentato una significativa ed indipendente associazione tra epatopatia steatosica non alcolica (NAFLD) e AVS nella popolazione generale adulta. Attualmente non è noto se tale associazione esista anche nei pazienti affetti da diabete tipo 2, che è una patologia in cui NAFLD ed AVS sono molto frequenti. Abbiamo pertanto valutato se la NAFLD si associava ad una maggior frequenza di AVS in pazienti affetti da diabete tipo 2.

Materiali e Metodi. Sono stati studiati 180 pazienti consecutivi affetti da diabete tipo 2, (M/F=135/45, età media ~69 anni, durata diabete ~15 anni), regolarmente afferenti presso il Servizio di Diabetologia, che risultavano clinicamente esenti da pregresso infarto miocardico, cardiomiopatia valvolare, epatopatia cronica ed abuso alcolico. In tutti i partecipanti, la presenza di NAFLD è stata accertata mediante ecografia epatica mentre quella di AVS è stata valutata mediante ecocardiografia doppler (eseguita da un unico operatore).

Risultati. Nel campione esaminato, la NAFLD era presente in 120 (66.7%) pazienti mentre AVS era presente in 53 (29.4%) pazienti. Nessun paziente aveva stenosi valvolare aortica. La presenza di NAFLD era significativamente associata ad un aumentato rischio di AVS (odds ratio [OR] 2.79, 95% CI 1.3-6.1, P<0.01). Tale associazione rimaneva significativa (adjusted-OR 2.68, 95% CI 1.2-6.1, P=0.01) dopo aggiustamento statistico per età, sesso, durata di diabete, fumo, HbA1c, parametri di funzionalità renale, ipertensione arteriosa e dislipidemia.

Conclusioni. I risultati di questo studio indicano che in pazienti ambulatoriali affetti da diabete tipo 2 la presenza di NAFLD si associa ad un'aumentata prevalenza di AVS, indipendentemente dai principali fattori di rischio cardiovascolare. Ulteriori studi sono necessari per confermare tali osservazioni e per definire i possibili meccanismi eziopatogenetici di tale associazione.

EPATOPATIA STEATOSICA NON ALCOLICA ED AUMENTATA INCIDENZA DI INSUFFICIENZA RENALE CRONICA IN PAZIENTI CON DIABETE TIPO 1

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Premessa e Scopo dello Studio. Recenti studi hanno evidenziato che l'epatopatia steatosica non alcolica (NAFLD) si associa ad un'aumentata incidenza di malattia renale cronica (CKD) nella popolazione generale e nei pazienti affetti da diabete tipo 2. Attualmente non sono disponibili dati sul possibile impatto prognostico della NAFLD nel diabete tipo 1. Lo scopo dello studio è stato quello di valutare se la NAFLD si associava ad un aumentato rischio di CKD in una coorte di diabetici tipo 1.

Materiali e Metodi. Sono stati arruolati tutti i diabetici tipo 1 ambulatoriali (n=261, 55% femmine, età 42±13 anni, durata diabete 20±12 anni), regolarmente afferenti presso il Servizio di Diabetologia nel periodo 1999-2001, che risultavano esenti da neoplasia, epatopatia cronica, abuso alcolico e CKD (definita come filtrato glomerulare stimato [eGFR] £60 ml/min/1.73 m² e/o macroalbuminuria). La NAFLD è stata accertata mediante ecografia epatica in tutti i partecipanti. I nuovi casi di CKD sono stati definiti come comparsa di e-GFR £60 ml/min/1.73 m² e/o macroalbuminuria durante il follow-up.

Risultati. Al baseline, i 261 partecipanti avevano un e-GFR medio di 92±23 ml/min/1.73 m² (stimato con formula MDRD); 234 (89.7%) erano normo-albuminurici e 27 (10.3%) erano microalbuminurici. La NAFLD era presente in 131 (50.2%) partecipanti. Durante un follow-up medio di 5.2±1.7 anni, 61 pazienti sviluppavano CKD (28 sviluppavano eGFR <60 ml/min/1.73 m² con micro-/macroalbuminuria, 21 sviluppavano eGFR ridotto con normoalbuminuria e 12 sviluppavano macroalbuminuria con eGFR conservato). La presenza di NAFLD si associava ad un' aumentata incidenza di CKD (hazard ratio [HR] 2.85; 95%CI 1.59-5.10, P<0.001). Tale associazione rimaneva significativa (adjusted HR 2.40; 95%CI 1.31-4.41; P<0.01) anche dopo aggiustamento per età, sesso, durata di diabete, HbA1c ed ipertensione arteriosa. I risultati rimanevano invariati anche dopo esclusione dei pazienti con microalbuminuria al baseline (adjusted HR 2.03; 95%CI 1.04-3.94; P<0.05).

Conclusioni. I risultati di questo studio indicano che in pazienti ambulatoriali adulti affetti da diabete mellito tipo 1 la presenza di NAFLD, documentata mediante ecografia epatica, si associa indipendentemente ad un' aumentata incidenza di CKD. Ulteriori studi prospettici sono necessari per confermare tali osservazioni.

EVALUATION OF A METHOD FOR THE INCORPORATION OF CHOLESTEROL FROM THE BACTERIAL STRAINS

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Background. The administration of probiotics in hyperlipidemic patients can influence the absorption of exogenous cholesterol, because these strains of bacteria can capture it and decrease its availability for absorption by the intestine.

By combining a drug therapy with the administration of probiotics it is possible to increase the lipid-lowering effect of bacteria and concomitantly to decrease the drug dosage, thus obtaining a reduction in the side-effects and an increase in patients' compliance. The purpose of this study is to standardize an in vitro method for the evaluation of the bacteria uptake of cholesterol, to use it in future as a screening method aimed to select the most active bacteria strains. Second we compared the cholesterol uptake between *Bifidobacterium bifidum* PRL2010 and *Lactobacillus helveticus*.

Methods. Probiotics were cultivated in Man Rogosa Sharpe medium (MRS) in the presence or absence of Ox-gall (bovine bile) and unesterified cholesterol, then radiolabeled with 1 µCi/ml of 3H-cholesterol and incubated at 37°C under anaerobic conditions. The quantification of the captured radioactive cholesterol occurred after bacteria lysis and has been normalized respect to the number of bacteria.

Results and Discussion. The presence of ox-gall in the medium reduced the uptake of 3H-cholesterol: 1.662% vs 3.903% cpm/108 bacteria respectively in presence and absence of this factor. In ad-

dition, an important variable is incubation time with the isotope for 18 h in growth phase favors the cholesterol uptake in the bacteria, which was insufficient with incubation for 4h. Finally, we applied this method to the comparison of the activity of cholesterol uptake of two different strains, *Bifidobacterium bifidum* PRL2010 and *Lactobacillus helveticus*. The second showed an activity of 5 times higher: 0.12% vs 0.53% cpm/108bacteria. In conclusion, with these preliminary experiments we optimize the method that can be used to perform screening of bacteria cholesterol uptake activity.

IPERLALIPOPROTEINEMIA: ANALISI GENETICA NEI SOGGETTI DELLO STUDIO ALEA

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Introduzione. Sono ancora discussi i rapporti tra livelli molto elevati di cHDL e aterosclerosi subclinica. Lo studio ALEA (iperAlfaLipoproteinemia e Aterosclerosi) si è prefisso l'obiettivo di analizzare i principali geni candidati in giovani adulti con iperalfalipoproteinemia (IAL, cHDL superiore al 95° percentile in assenza di cause secondarie riconoscibili), e di determinare i rapporti tra IAL, distribuzione delle sottofrazioni lipoproteiche, parametri infiammatori, efflusso cellulare di colesterolo e indici di malattia vascolare subclinica. Verranno qui riassunti i dati della determinazione genetica.

Materiali e Metodi. È stata condotta l'analisi dei geni CETP, SRB1 e LIPG, attraverso un sequenziamento diretto delle regioni codificanti e dei confini fra introni ed esoni di 20 soggetti arruolati nello studio.

Risultati. Il sequenziamento genetico del gene CETP ha permesso l'identificazione di un nuovo Polimorfismo a Singolo Nucleotide (SNP, Asp131Asn), probabilmente patogenetico, ed inoltre di due soggetti portatori rispettivamente della mutazione p.Ala390Pro (Minor Allele Frequency, MAF=0.033) e p.Arg468Gln (MAF=0.030), che vengono riportati come polimorfismi "rari" di incerto significato. È stata inoltre osservata una significativa maggior prevalenza del comune SNP p.Val422Ile (50% vs 27.4% in un gruppo di 200 soggetti normolipemici di controllo). La percentuale di soggetti portatori di almeno un allele "non comune" (modello dominante) era 0.85 nel gruppo IAL e 0.43 in quello di controllo (p=0.003). Il sequenziamento del gene SR-B1 ha rilevato la presenza del polimorfismo noto p.Gly2Ser (MAF=0.077) in eterozigosi (3 soggetti), quello del gene LIPG gene ha condotto all'identificazione di 6 portatori del comune polimorfismo p.Thr111Ile (MAF=0.244) ed 1 della rara variante p.Asn396Ser (MAF=0.005), potenzialmente patogenetica.

Conclusioni. L'analisi qui riportata fornisce alcuni indizi relativi alla base genetica della IAL che riconosce, almeno nella nostra popolazione e in rapporto ai geni esaminati, un ruolo preminente di polimorfismi comuni rispetto a mutazioni rare ma funzionalmente attive.

GENETIC CHARACTERIZATION OF TWO NOVEL CASES OF CHOLESTERYL ESTER STORAGE DISEASE (CESD)

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CESD is a recessive disorder due to mutations of the LIPA gene encoding LAL (Lysosomal Acid Lipase). Recently we genetically characterized two novel probands affected to CESD:

Family 1. The proband was a 2 year-old Greek male (BMI 15.7 kg/m²) with dyslipidemia (TC 349, LDL-C 280, HDL-C 24, TG 150, ApoA1 82, ApoB 174 mg/dl) mild hepatomegaly and increased levels of transaminases (AST 59, ALT 83 U/l). Antibodies for EBV, CMV, SMF, LKM, P-ANCA, C-ANCA, Gliadin (IgA, IgG), endomysium, tTG, ANA, Anti-DNA were negative. LAL activity in leukocytes was 9% of normal. The sequence of LIPA gene revealed that patient was a compound heterozygous for the more common c.894G>A, p.(S275 Q298del) CESD mutation and for c.1024G>A, p.(G342R) mutation, which we previously found in another compound heterozygous CESD patient from Greece.

Family 2. The proband was a Croatian-Australian male, who at the age of 4, was referred to Hospital for lethargy, anorexia, failure to thrive (BMI 14.8 kg/m²), fever, night sweats and hepatomegaly. Serology showed AFP, HCG, Hep A/B/C, EBV, VZV, HSV negative, past infection of CMV. At the age of 12 clinical and biochemical examination revealed AST 159, ALT 198, GGT 155 U/L, Cholesterol 313, HDL-C 25, TG 184 mg/dl and adrenal insufficiency. LAL activity in blood leukocytes was 2.9% of normal. Liver biopsy demonstrated fat infiltration in enlarged vacuolated hepatocytes & Kupffer cells. By sequencing LIPA gene we found that patient was compound heterozygous for c.894G>A, p.(S275-Q298del) CESD mutation and c.419G>A, p.(W140*) we previously found in a homozygous Croatian child with Wolman disease (WD). The patient now is enrolled in a clinical trial with human recombinant LAL. The two uncommon mutations co-segregated with the two different haplotypes that we previously reported in the Greek CESD and in the Croatian WD, suggesting common ancestors in these Countries.

INCREASED NOX2 GENERATED OXIDATIVE STRESS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aim. Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver diseases ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis and even cirrhosis and hepatocellular carcinoma. Chronic oxidative stress is considered one of the key mechanisms responsible for liver damage and disease progression in non-alcoholic fatty liver disease. However, so far, no study has

been performed with newer markers of systemic oxidative stress. Aim of this study was to assess the relationship between urinary 8-iso-prostaglandin F₂α (8-iso-PGF₂α) and serum soluble NOX2-derived peptide (sNOX2-dp) and the severity of liver steatosis in subjects with non-alcoholic fatty liver disease in different clinical settings.

Methods. The study has been performed in 264 consecutive patients referred for suspected metabolic disease. Metabolic syndrome was diagnosed according to the modified criteria of the ATP III Expert Panel of the US National Cholesterol Education Program. Liver steatosis was defined according to Hamaguchi ultrasonographic criteria. Oxidative stress was assessed by urinary 8-iso-PGF₂α and serum sNOX2-dp levels.

Results. Patients with NAFLD had significantly higher (p<0001) mean values of urinary 8-iso-PGF₂α, and serum sNOX2-dp, ALT, and cytokeratine-18 and HOMA-IR and lower values of serum adiponectin as compared to those without. Prevalence of MetS and of most of its clinical features was significantly higher in patients with NAFLD. The same findings were also observed after the exclusion of obese subjects, or subjects with diabetes or with metabolic syndrome and in those not taking statin medication. In addition, the levels of urinary 8-iso-PGF₂α were independent predictors of NAFLD and a strong association of urinary 8-iso-PGF₂α and of serum sNOX2-dp with the severity of liver steatosis at ultrasound examination was also observed.

Conclusions. We demonstrated an increased NOX2 generated oxidative stress in subjects with NAFLD. Oxidative stress was independent from obesity, diabetes and metabolic syndrome and increased with the severity of liver steatosis at ultrasound.

KRP-203, SPHINGOSINE 1-PHOSPHATE RECEPTOR TYPE 1 AGONIST, AMELIORATES ATHEROSCLEROSIS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT (LDL-R-/-) MICE

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Objective. Sphingosine 1-phosphate (S1P) partly accounts for antiatherogenic properties of high-density lipoproteins. We previously demonstrated that FTY720, a synthetic S1P analog targeting all S1P receptors but S1P receptor type 2, inhibits murine atherosclerosis. Here, we addressed the identity of S1P receptor mediating atheroprotective effects of S1P.

Approach and Results. Low-density lipoprotein receptor-deficient mice on cholesterol-rich diet were given selective S1P receptor type 1 agonist KRP-203 (3.0 mg/kg per day; 6 and 16 weeks). KRP-203 substantially reduced atherosclerotic lesion formation without affecting plasma lipid concentrations. However, KRP-203 induced lymphopenia, reduced total (CD4(+), CD8(+)) and activated (CD69(+)/CD8(+), CD69(+)/CD4(+)) T cells in peripheral lymphoid organs, and interfered with lymphocyte function, as evidenced by decreased T-cell proliferation and interleukin-2 and interferon-γ production in activated splenocytes. Cyto- and chemokine (tumor necrosis factor-α, regulated and normal T cell expressed and secreted) levels in plasma and aortas were reduced by KRP-203 administration. Moreover, macrophages from KRP-

203-treated mice showed reduced expression of activation marker MCH-II and poly(I:C)-elicited production of tumor necrosis factor- α , monocyte chemoattractant protein-1, and interleukin-6. In vitro studies demonstrated that KRP-203 reduced tumor necrosis factor- α , interleukin-6, and interferon- γ -induced protein-10 production; I κ B and signal transducer and activator of transcription-1 phosphorylation; and nuclear factor κ B and signal transducer and activator of transcription-1 activation in poly(I:C)-, lipopolysaccharide-, or interferon- γ -stimulated bone marrow macrophages, respectively.

Conclusions: Present results demonstrate that activation of S1P signaling pathways inhibit atherosclerosis by modulating lymphocyte and macrophage function and suggest that S1P receptor type 1 at least partially mediates antiatherogenic effects of S1P.

EFFETTI DEL LDL-AFERESI NELLA GLOMERULOPATIA DA LIPOPROTEINE

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Introduzione. La glomerulopatia da lipoproteine, descritta da Saito nel 1987, è caratterizzata da depositi di lipoproteine nel lume dei capillari glomerulari, da iperlipoproteinemia di tipo III ed elevati livelli sierici di apolipoproteina E. Si tratta di una malattia estremamente rara e a più alta prevalenza in soggetti di origine asiatica. Si manifesta con sindrome nefrosica ad evoluzione naturale verso l'insufficienza renale terminale.

Quadro clinico. Due soggetti con diagnosi istologica di glomerulopatia da lipoproteine (presenza alla biopsia renale di depositi di lipoproteine nel lume capillare glomerulare associati a dilatazione dei glomeruli sono stati riferiti al nostro Centro per le Dislipidemie Ereditarie.

Il primo paziente è un soggetto di 44 anni di razza caucasica che in corso di terapia con ACE inibitori, sartanici ed ipolipemizzanti (simvastatina/ezetimibe), presentava progressivo peggioramento della funzionalità renale (creatinemia 1,6 mg/dl; proteinuria 9,9 g/24 ore) e scarso controllo circadiano della pressione arteriosa. Nonostante un soddisfacente assetto lipoproteico, alla terapia farmacologica è stato affiancato il trattamento con LDL-afèresi ad intervalli bisettimanali, trattando mediamente 4000 cc di plasma in 3 ore. Ad oggi sono stati effettuati 45 trattamenti di LDL-afèresi in assenza di effetti collaterali. Progressivamente si è assistito alla stabilizzazione ed alla normalizzazione dei valori di pressione arteriosa, alla riduzione dei livelli di proteinuria (fino a 1,6 g/24 ore già dopo 10 trattamenti), al miglioramento della funzione renale (filtrato glomerulare 58 ml/min - sec. Cockcroft-Gault normalizzato per la superficie corporea), all'incremento della proteinemia totale ed alla riduzione di peso corporeo (da 76,1 a 67,4 kg).

Il secondo paziente è un soggetto di 23 anni di razza caucasica che in corso di terapia con ACE inibitori, sartanici e calcioantagonisti presentava progressivo peggioramento della funzionalità renale (creatinemia 1,4 mg/dl; proteinuria 16,4 g/24 ore) e scarso controllo circadiano della pressione arteriosa. In relazione alla severità della sindrome nefrosica è stata intrapreso trattamento di LDL-afèresi, ottenendo un progressivo miglioramento clinico e del quadro nefrosico (proteinuria 2,4 g/24 ore dopo i 27 trattamenti ad oggi effettuati).

Conclusioni. I casi di cui sopra si aggiungono alla piccola coorte, in maggioranza di soggetti asiatici, sin qui descritta. Il primo

soggetto è caratterizzato dall'assenza di un profilo lipidico "tipico" di questi casi (profilo simil-iperlipoproteinemia di tipo III ed elevata concentrazione di apolipoproteina E).

In entrambi i casi la risposta al trattamento di LDL-afèresi, anche se ottenuta con tempi diversi, non sembra imputabile al solo miglioramento del profilo lipidico ma plausibilmente potrebbe, anche solo parzialmente, essere spiegata con il ripristino della funzione endoteliale indotta dal trattamento di LDL-afèresi.

METABOLIC CONSEQUENCES OF ADIPOSE TRIGLICERIDE LIPASE DEFICIENCY IN HUMANS: AN IN VIVO STUDY IN PATIENTS WITH NEUTRAL LIPID STORAGE DISEASE WITH MYOPATHY

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Background. The role of adipose triglyceride lipase (ATGL) in intermediate substrates metabolism has not been fully elucidated in humans. Neutral Lipid Storage Disease with Myopathy (NLSMD), a rare disorder caused by inactivating mutations of the ATGL-coding gene represents an unique model to gain further insight on this metabolic pathway.

Aim. The aim of the present study was to use state-of-the-art techniques to determine the metabolic consequences of global ATGL deficiency in humans including body fat distribution and tissue fat content, cardiac function and metabolism, tissue-specific and whole body insulin sensitivity and energy substrate metabolism.

Methods. Three patients affected by NLSMD due to homozygosity for loss-of-function mutations in the ATGL gene and 6 sex-, age-, and body mass index-matched controls were studied. Body composition and organ fat content were measured by bioimpedance and (1)H nuclear magnetic resonance spectroscopy; heart glucose metabolism by [(18)F]deoxyglucose positron emission tomography and insulin sensitivity and β -cell function by oral glucose tolerance and 2-step euglycemic-hyperinsulinemic clamp. Lipolysis [(2)H5] glycerol turnover) and indirect calorimetry were evaluated at fasting, after oral glucose load, during the clamp, and also during an iv epinephrine infusion. These metabolic investigations were carried out during hospitalization.

Results. As expected, NLSMD patients showed diffuse, although heterogeneous, fat infiltration in skeletal muscles associated with increased visceral fat. Although heart and liver were variably affected, fat content in the pancreas was increased in all patients. Compared with healthy controls, NLSMD patients showed impaired insulin response to glucose possibly related to the severe pancreatic steatosis, preserved whole-body insulin sensitivity, and a shift toward glucose metabolism in the heart. Fasting non-esterified fatty acid concentrations as well as basal lipolytic rates and the anti-lipolytic effect of insulin were normal in NLSMD patients, whereas the lipolytic effect of norepinephrine was impaired. Finally, no significant abnormality in the respiratory quotient was noted in NLSMD patients.

Conclusions. In humans, ATGL has a remarkable effect on cel-

lular lipid droplet handling, and its lack causes both perivisceral, skeletal muscle, and pancreas fat accumulation; in contrast, the impact on whole-body insulin sensitivity and fatty acid metabolism is minor.

SEVERE HYPERTRIGLYCERIDEMIA IN TWO NEWBORNS DUE TO NOVEL MUTATIONS IN LPL AND APOC2 GENES

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Background. Severe hypertriglyceridemia (sHTG) (plasma TG >10 mmol/L) in newborns may be due to monogenic defects of the lipolytic cascade, the process by which chylomicron and VLDL triglycerides are hydrolyzed by Lipoprotein Lipase (LPL). Mutations in any of the five candidate genes (LPL, APOC2, APOA5, GPIIIBP1 and LMF1) may be the cause of sHTG.

Methods. The candidate genes were resequenced in two newborns with sHTG and at risk of acute pancreatitis.

Results. The first child was admitted to the hospital for the presence of milky serum and the following lipid profile: TC 17 mmol/L and TG 110 mmol/L with chylomicronemia but no signs of pancreatitis or other clinical manifestations. He was homozygous for a nucleotide deletion in exon 6 (c.840delG) of LPL gene resulting in a truncated LPL of 302 amino acids, devoid of function. This mutation was found in the mother and one sister of the patient but not in his father. The latter turned out to be a carrier of a deletion eliminating the entire LPL gene. The patient was therefore a compound heterozygote (nucleotide deletion in exon 6/deletion of LPL gene). Both mutations are novel.

The second child was a female admitted to hospital for acute pancreatitis and plasma TG >200 mmol/L. She was found to be homozygous for a dinucleotide deletion (c.196-197del GC) in exon 3 of APOC2 gene, resulting in a truncated protein of 46 amino acids, devoid of the lipid binding C-terminal domain. In addition she had a lipid deposit in the right hemisphere, as previously reported in another child with APOC2 deficiency.

Conclusions. The presence of sHTG in a newborn, even in the absence of overt clinical manifestations should suggest the presence of a monogenic defect in the lipolytic cascade.

LP(A), LIPOPROTEINE ED ETÀ: IMPATTO SULL'ARTERIOPATIA CAROTIDEA

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L'aterosclerosi è una patologia multifattoriale che inizia in età precoce e si manifesta nella media e terza età. Da 50 anni è stato individuato un nuovo marker di rischio vascolare: la Lp(a), costituita da una LDL legata mediante ponte di solfuro alla glicoproteina apo(a). Il ruolo patogenetico della Lp(a) è ancora oscuro, sebbene le sia stata attribuita una funzione proaterogena e protrombotica. Pochi lavori hanno correlato l'iperLp(a) con l'arteriopatia carotidea.

La nostra ricerca ha lo scopo di valutare l'impatto dell'Lp(a) sull'arteriopatia carotidea.

Sono stati studiati 1013 soggetti, 498 M e 515 F (età media 67.5±12.3 anni) tutti sottoposti ad anamnesi personale e familiare, esame obiettivo, esami ematochimici e strumentali, accertando le seguenti condizioni: abitudine tabagica, ipertensione, coronaropatie, diabete mellito, ictus ischemico, arteriopatia carotidea. Sono stati considerati affetti da arteriopatia carotidea i pazienti con IMT > 0.9 mm.

È risultato che i pazienti con stenosi carotidea hanno valori maggiori di età (p<0.0005), di colesterolo totale (p<0.0005), di colesterolo nonHDL (C-nonHDL) (p<0.0005), di colesterolo LDL (C-LDL) (p<0.001), di trigliceridi (p<0.0005), di Apo B (p<0.0005), di Lp(a) (p<0.0005), dei rapporti C-nonHDL/C-HDL (p<0.002), C-LDL/C-HDL (p<0.003) ed ApoB/ApoAI (p<0.0005).

Dall'analisi multivariata si conferma che con l'età aumenta sensibilmente la probabilità di stenosi carotidea (p<0.0005). L'ipertensione, il fumo e il diabete mellito non appaiono incidere significativamente sulla probabilità di arteriopatia carotidea. Elevati valori di C-nonHDL (p<0.0005), di Apo B (p<0.0005), dei rapporti C-nonHDL/C-HDL (p<0.0005) e Apo B/Apo A-I (p<0.0005), e bassi valori di C-HDL (p<0.07) e di Apo A-I (p<0.04) incrementano fortemente il rischio di arteriopatia. I pazienti con Lp(a) >30 mg/dL hanno una probabilità quattro volte maggiore di sviluppare l'arteriopatia carotidea (Odds Ratio: 4.335, 95% IC 2.850-6.593, p<0.0005).

In conclusione, l'età, il C-nonHDL, l'Apo B e, soprattutto, la Lp(a) in modo indipendente sono markers di rischio di arteriopatia carotidea.

PREDITTORI DI DANNO VASCOLARE NEL GRANDE OBESO SOTTOPOSTO A CHIRURGIA BARIATRICA

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Background e Scopo dello Studio. La disfunzione endoteliale, espressione di danno vascolare precoce, si associa all'obesità d'alto grado (BMI ≥35-40 kg/m²). Recenti studi evidenziano però che, dopo chirurgia bariatrica o "chirurgia metabolica", la riduzione maggiore del rischio cardiovascolare si osserva in coloro che presentavano valori di insulinemia pre-intervento più elevati. Scopo del nostro studio è valutare le modificazioni metaboliche e vascolari dopo sleeve-gastrectomy, con particolare attenzione a due parametri: body mass index (BMI), espressione di adiposità generalizzata, ed HOMA-IR, indicatore di insulino-resistenza.

Pazienti e Metodi. In 43 soggetti (BMI medio 44 kg/m²) abbiamo determinato glicemia, insulinemia, HOMA-IR, lipidi, grasso viscerale (visceral fat area, VFA) e vasoattività flusso-mediata (VFM) prima e dopo 10 mesi dall'intervento.

Risultati. Dopo l'intervento si è osservata una riduzione di BMI (da 44 a 32 kg/m², p<0.001), HOMA-IR (da 4 a 1.5, p<0.001), VFA (da 248 a 148 cm², p<0.001), mentre la VFM è incrementata (da 10 a 13%, p=0.037). Suddividendo i soggetti in due gruppi sulla base della mediana dell'HOMA-IR pre-intervento (< e ≥3.5), abbiamo osservato in entrambi, dopo chirurgia, una riduzione significativa della VFA, del grasso sottocutaneo e dei valori pressori, mentre un incremento significativo della VFM si è osservato solo nei soggetti con HOMA-IR ≥3.5. Non sono invece emerse differenze significa-

tive di VFM prima e dopo l'intervento suddividendo i pazienti sulla base della mediana del BMI.

Discussione. Il significativo miglioramento della funzione endoteliale dopo chirurgia bariatrica, evidente solo nei soggetti con HOMA-IR pre-intervento più elevato, rafforza l'ipotesi dell'insulino resistenza e del grasso viscerale come determinanti di danno vascolare anche nel grande obeso. Il BMI, espressione di adiposità generale e determinante nel grande obeso di multiple e gravi comorbidità, sembra rivestire un ruolo di minor importanza nel danno vascolare.

VASCULAR GENETIC PROFILE IN WOMEN WITH HISTORY OF ADVERSE PREGNANCY OUTCOMES: ROLE OF ACE AND ENOS GENES IN STILLBIRTH

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Introduction. Beyond traditional cardiovascular risk factors, clinical studies indicate that a history of placenta-mediated pregnancy complications increases future cardiovascular risk in women (1). A history of preeclampsia as well as spontaneous recurrent miscarriage and stillbirth (2), increases the risk of cardiovascular disease (3) later in life, possibly related to the persistence of endothelial dysfunction which is responsible for placentation-related defects, thus representing the link between pregnancy complications and future vascular events (4). We investigated a possible common genetic background shared by women with history of placenta-mediated pregnancy complications (PMPC) and premature cardiovascular events (CVD).

Materials and Methods. We investigated 367 women with history of PMPC [147 small for gestational age (SGA), 176 preeclampsia (PE), and 117 stillbirth (SB)], 290 with premature CVD, and 300 HW referred to Gender Medicine Clinic of the Atherosclerotic Disease Center, Department of Experimental and Clinical Medicine, Florence. All women were analyzed for ACE I/D and -240A>T, AGT M235T, AGTR1 1166A>C and eNOS -786T>C, 894G>T, and 4a/4b polymorphisms.

Results. In PMPC, ACE D allele frequency was comparable with that observed in premature CVD group, and higher than that observed in HW [OR(95%CI) 2.18 (1.47-3.22) p<0.0001 and 1.91 (1.27-2.88) p=0.002, respectively]; by analyzing each PMPC group, similar D allele frequency was found. In women carrying ACE-240T or eNOS-786C allele a two-fold increase in SB susceptibility was evidenced [OR(95%CI) 2.25 (1.37-3.72), p=0.001] and 1.81 (1.10-3.00) p=0.02, respectively).

Our results provide evidence of a common genetic background shared by women with history of PMPC and premature CVD, and highlights the involvement of both ACE and eNOS genes in stillbirth susceptibility.

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SCREENING OF APOB AND PCSK9 GENES IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS WITHOUT MUTATIONS IN THE LDLR GENE

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Introduction. Familial hypercholesterolemia (FH) is a genetically heterogeneous lipid disorder with a frequency of 1:500 for heterozygotes. FH is caused by mutations in the LDL receptor (LDLR, 73-76%), in the Apolipoprotein B (ApoB, 3-6%), and in the Proprotein Convertase, Subtilisin/Kexin-type 9 (PCSK9, less than 1%) genes. We aim to enlarge the genetic spectrum of FH mutations in the APOB and PCSK9 genes.

Materials and Methods. We selected 81 patients with a clinical diagnosis of FH but no detected mutations in LDLR gene. The APOB screening included the extended binding region of the gene (codons 3182-3929 in the exon 26 and codons 4030-4563 in the exon 29) whereas PCSK9 screening was performed in 50 patients through direct sequencing of the 12 exons with flanking intron sequences.

Results. In APOB gene we found two variants one of which is new and in PCSK9 gene we identified only one mutation. The new genetic variant (c.10897T>C) identified in APOB gene causes the aminoacid change p.Tyr3633Arg. The presence of the variant was excluded in 180 chromosomes from healthy subjects. The tyrosine at the position 3633 was conserved in the 20 analyzed species. In silico analysis revealed that the substitution is "Not tolerated", "Pathological", "Probably damaging" and "Polymorphism" according to the algorithm SIFT, PMut, Poly-Phen and Mutation Taster, respectively.

Conclusions. Our results show that the percentage of mutations in APOB and PCSK9 is in agreement with results reported in other population suggesting that the screening of the APOB and PCSK9 genes should be included in the diagnostic procedures, although the mutation frequency is low.

SMOKE EXPOSURE PRECOCIOUSLY AFFECT CARDIAC PERFORMANCE IN YOUNG HEALTHY SMOKERS

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Little is known on the early impact of smoking on cardiac function in young smokers. The new complementary echocardiography speckle-tracking (STE) software adds specific information on left

ventricular (LV) global strain (GS) and torsion providing a non invasive estimate on LV systolic deformation. We investigated the subclinical LV dysfunction by STE in active cigarette smokers with no additional risk factors. Fifty smokers (mean age 24±5 years) and 60 matched controls were enrolled for this study. Plasma concentration of lipids, glucose, fibrinogen, C-reactive protein (CRP), interleukin-6 (IL-6), and blood pressure values were evaluated. Arterial stiffness indices (AIx and PWV), carotid intima-media thickness (cIMT) values and echocardiographic parameters were also evaluated. Left ventricular GS (longitudinal, radial and circumferential strain) and torsion were measured at rest and during exercise (STE software: GE, EchoPac-v.7.0.0). To evaluate the influence of smoking on study variables a score of smoke exposure was estimated (SEIx). With respect to controls fibrinogen ($p<0.05$), CRP ($p<0.01$), IL-6 ($p<0.01$) and arterial stiffness (AIx: $p<0.05$; PW $p<0.01$) were higher in smokers while HDL-C ($p<0.01$) was lower. No difference was found in cIMT values between smokers and controls. At rest, GS and torsion were not different with respect to controls. During exercise, we found worse values of GS and torsion at 50w ($p<0.001$ and $p<0.05$, respectively), at 100w ($p<0.05$ and $p<0.001$, respectively), and of torsion at recovery ($p<0.001$) with respect to controls. Regression analysis indicated a significant association between SEIx and HDL, between HDL and inflammatory markers and between inflammatory markers and AIx, PWV, GLS at 50w and torsion at recovery. These findings provide a model according to which in young smokers occurs a slower adaptation to physical effort and confirm that smoking affects HDL concentration inducing a pro-inflammatory status. Furthermore we suggest that inflammation in smokers may precociously influence cardiac function.

GLP-1 INFLUENCES THE INHIBITORY EFFECTS OF NITRIC OXIDE ON PLATELETS

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Background and Aims. Glucagon-like peptide-1 (GLP-1) exerts cardiovascular effects, sometimes independently of GLP-1 receptor (GLP-1R). GLP-1 effects on platelets, however, have not been investigated. After its secretion, native GLP-1 amide is degraded to the main circulating form GLP-1(9-36) amide, unable to interplay with GLP-1R. We aimed to investigate the effects of GLP-1(7-36), GLP-1(9-36) amides and GLP-1 analogue Liraglutide on platelet aggregation and sensitivity to the anti-aggregating pathway nitric oxide (NO)/cGMP/VASP and to clarify whether the effects on platelets are GLP-1R dependent.

Materials and Methods. In platelets from 38 healthy subjects (M/F 23/15; age: 22.7±6.6 years; body mass index: 21.6±2.3 kg/m²) we measured the effects of a 10-min pre-incubation with 100 nmol/l of GLP-1(7-36), GLP-1(9-36) and Liraglutide on:

- 1) platelet aggregation to collagen (4 mg/l),
- 2) cGMP synthesis (ELISA),
- 3) expression of pVASP-ser-239 (WB), with or without the NO donor sodium nitroprusside (SNP) (1-5 nmol/l, 5 min). Experiments were repeated with the GLP-1R antagonist Exendin (9-39) (100 nmol/l).

Results. GLP-1(7-36), GLP-1(9-36) and Liraglutide did not affect platelet aggregation, cGMP secretion and VASP expression, but increased the inhibitory effects of SNP. In particular, i) percent inhibition of collagen-induced aggregation with SNP alone, SNP+GLP-1(7-36), SNP+GLP-1(9-36) and SNP+Liraglutide was

35.1±12.6, 62.1±13.6, 60.7±12.4 and 50.7±21 ($p<0.0001$ vs SNP alone for all); ii) percent increase on baseline of cGMP concentrations with SNP alone, SNP+GLP-1(7-36) and SNP+GLP-1(9-36) was 92.6±42.8, 171.1±80.2 ($p<0.01$ vs SNP) and 197.3±92.9 ($p<0.04$ vs SNP); iii) percent increase of pVASP ser-239 expression with SNP alone, SNP+GLP-1(7-36), SNP+GLP-1(9-36) and SNP+Liraglutide were 198.6±74.5, 371.1±94.4 ($p<0.001$ vs SNP), 423±93.9 ($p<0.0001$ vs SNP), and 398.6±75.7 ($p<0.005$ vs SNP), respectively. Pre-incubation with Exendin(9-39) did not modify the effects of the incretin preparations on the platelet inhibitory effects of SNP, indicating that they are independent on GLP-1R.

Conclusions. In platelets from healthy subjects high concentrations of GLP-1 increase platelet sensitivity to NO via GLP-1R-independent mechanisms.

CHARACTERIZATION OF MEMORY T CELL SUBSETS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Chronic inflammatory process, involving both innate and adaptive immune system cells, appears to be a key determinant in atherosclerosis development. T lymphocytes are detected into atherosclerotic lesions during all stages of the disease suggesting that they may be involved in the initiation and progression of atherosclerotic plaques.

Recent studies both on atherosclerosis animal models (ApoE KO and LDLR KO mice) and on humans, showed increased prevalence of effector memory T cells in conditions associated to pre-clinical atherosclerosis, in patients with chronic stable angina and myocardial infarction, probably due to the fact that antigens generated as a consequence of hypercholesterolemia and presented by antigen-presenting cells stimulate the development of central memory T cells (TCM) and effector memory T cells (TEM), from Naïve (TN) cells. To further exploit these observations, we aimed at characterizing the distribution of memory T cell subsets in subjects affected by familial hypercholesterolemia (FH), a condition linked to early atherosclerosis. The percentages of naïve and memory T cells (out of CD4+ cells) were investigated in 76 Heterozygous FH patients and 92 age/sex matched control individuals by flow cytometric analysis. Memory T cell subsets (CD4+ CD45RA-) were further identified as central memory (CCR7+) and effector memory (CCR7-) T cells. FH patients present with a significant increased of CD4+ effector memory T cells (21,64±9,11% vs 18,16±6,82%, $p=0.004$) compared to age and sex matched controls. Studies are on-going to investigate whether TEM cells could promote and support the increased immuno-inflammatory responses in FH or represent a consequence of the disorder. In summary, we have demonstrated that in FH subjects there is prevalence of effector memory T cells suggesting a role of hypercholesterolemia in the modulation of this important pathway.

EFFECTS OF ROSUVASTATIN ON THE EXPRESSION OF MICRORNAS AND THEIR PUTATIVE TARGETS IN HUMAN ATHEROSCLEROTIC PLAQUES: THE QUASAR STUDY

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Background. Plenty of evidence supports a role of statin on stabilization of atherosclerotic plaque in animal models and in human studies. MicroRNAs are a class of non-coding RNA which act as post-transcriptional modulator of gene expression. Their involvement in atherosclerosis is supported by in vitro, in animal, and human studies. We have previously found that microRNAs are expressed in human atherosclerotic plaques and that they impact the expression of key genes for plaque stability. The aim of this study is to investigate whether treatment with low- or high-dose of rosuvastatin may exert plaque-stabilizing actions via modulation of microRNA expression in human atherosclerotic plaques.

Material and Methods. 70 patients with severe asymptomatic carotid stenosis were randomized to receive a 12 week low (10 mg/day) or high (40 mg/day) doses of rosuvastatin before the elective endarterectomy. Pooled samples from 11 plaques of patients treated with rosuvastatin 10 mg and 40 mg groups and from 11 plaques of naive hypercholesterolemic patients (control group) were used for a preliminary screening. Then, the levels of differentially expressed microRNAs were validated in the wider population. Predicted target genes were estimated by using miR-Base and miRanda, and their expression was evaluated by qPCR and Western Blot.

Results. miRNome qPCR analysis of 742 microRNAs on pooled samples, and their subsequent validation on the first population showed that both rosuvastatin doses significantly up-regulated mir-9, mir-20b, mir-133a/b, mir-144, mir-301a, miR-222, and mir-377 ($p < 0.01$, for all) with respect to hypercholesterolemic patients. These results were confirmed in the second validation step on the wider population ($p < 0.05$, for all). Among these microRNAs, miR-133a/b was predicted to target Matrix Metalloproteinase (MMP)-9, whereas miR-301a was a putative modulator of PAI-1. MMP-9 gene expression showed an inverse correlation with their regulator miR-133a/b ($r = -0.362$ and $r = -0.397$, respectively, $p < 0.005$) whereas PAI-1 was inversely correlated with miR-301a ($r = -0.366$, $p = 0.002$). Finally, analysis of protein confirm the reduction of MMP-9 in atherosclerotic plaques from patients treated with rosuvastatin ($p < 0.02$). No significant differences were found in PAI-1 protein levels.

Conclusions. These results suggest that short-term rosuvastatin treatment may affect the expression of microRNAs and their putative target genes in human atherosclerotic plaques. This study point out a new potential mechanism of plaque stabilization for rosuvastatin.

NESFATIN-1 CORRELATED WITH THE RELATIONSHIP BETWEEN BIRTH WEIGHT AND WEIGHT AT THE 50TH PERCENTILE IN BABIES FROM GDM

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Introduction. Nesfatin-1 is a newly identified peptide involved in the regulation of food intake, body weight and insulin metabolism in rodents. There are few human studies and the results are controversial. To investigate the possible association of nesfatin-1 in the body weight of newborn or mother, we measured fasting serum mother nesfatin-1 concentrations in physiological or diabetic pregnant.

Methods. Thirty-nine pregnant women with GDM and thirty-two healthy pregnant women with similar ages and BMI comprised the study cohort. Fasting blood samples were obtained from mother at the day of delivery/birth. Nesfatin-1 was measured in serum with a commercial ELISA kit. Anthropometric data of babies were collected.

Results. Nesfatin-1 were not detected in 13 control sample (39.4%) and in 22 GDM samples (56.4%). Nesfatin-1 concentration were slightly lower but not significantly different in GDM group versus control group. Nesfatin-1 correlated with the relationship between birth weight and weight at the 50 th percentile (real/50th weight) in babies from GDM but not control mothers. Nesfatin-1 not correlated with BMI but correlated with weight before pregnancy of mother with BMI below 25.6 kg/m² instead in obese mother before pregnancy nesfatin-1 correlated with insulin levels and with the real/50th-weight in babies.

Conclusion. Our results suggest that nesfatin-1 is associated with weight in normal women and is altered in insulin resistance conditions like obesity and GDM where correlate with insulin levels and real/50 th - weight in newborn.

MEAN PLATELET VOLUME IN ACUTE CORONARY SYNDROME PATIENTS: ROLE OF A GENETIC VARIANTS IN CHR7Q22.3 AND CHR3P13-P21

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Several studies have demonstrated an association between elevated mean platelet volume (MPV), low platelet number (Plt) and coronary artery disease (CAD). Animal and twin studies demonstrated that MPV and Plt are genetically determined. Genome-wide association studies on healthy subjects identify several loci associated with MPV and Plt phenotypes, among which rs342293 polymorphism on chr7q22.3 and rs12485738 polymorphism on chr3p13-p21. No data are available on the possible association between these polymorphisms and platelet phenotypes in patients with CAD.

Aim of our study was to evaluate the association of these two polymorphisms with MPV and Plt in a cohort of acute coronary syndrome patients undergoing PCI with stent implantation (RE-CLOSE 2 ACS study).

We genotyped 1433 ACS patients by TaqMan technology and specific assay. The two polymorphisms were in Hardy-Weinberg equilibrium. The genotype distribution and minor allele frequencies were consistent with those reported in literature and dbSNP: rs342293 CC 36.1%, CG 46.4%, GG 17.5%, MAF G=0.407; rs12485738 GG 35.7%, GA 47.0%, AA 17.3% MAF A=0.408.

Concerning MPV and rs342293 polymorphism, in GG homozygous patients MPV values were significantly higher than in heterozygous and wild-type homozygous patients [9.3 (6.0-19.3) fL vs 8.8 (6.2-37.1) fL, $p=0.001$]. Concerning Plt and rs342293, patients carriers of the G allele showed lower Plt than CC wild-type homozygous patients [219 (80-805) platelets $\times 10^3/\text{microL}$ vs 226 (77-1360) platelets $\times 10^3/\text{microL}$, $p=0.038$]. No association between MPV, Plt and rs12485738 polymorphism was observed. At the linear regression analysis adjusted for age, sex, and Plt rs342293 polymorphism was a significant and independent predictor of MPV [Beta=0.230, standard error=0.113, $p=0.042$].

In conclusion, our results confirm and extend in ACS patients the role of rs342293 polymorphism on MPV phenotype.

ANEURISMA CORONARICO 20 ANNI DOPO "ROTABLATOR" IN SOGGETTO CON IPERCOLESTEROLEMIA FAMILIARE REFRATTARIA - LIPID LOWERING VERSUS REVASCULARIZATION THERAPY

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Introduzione. Nel 1997 J.S. Forrester et al., in un editoriale su *Circulation* "Lipid lowering versus revascularization therapy", sentenziavano che era arrivato il tempo per ottenere le prove definitive della superiorità della terapia ipolipemizzante sulle procedure di rivascularizzazione coronarica. Solamente due anni dopo B. Pitt et al., su *New England Journal of Medicine*, rafforzavano ulteriormente questa linea di pensiero affermando che

"High-quality lipid management is important, if not more important, than myocardial revascularization, often considered a more valuable aspect of cardiac care by many patients and physicians".

Storia clinica e follow-up. Un soggetto di 68 anni, maschio, caucasico, non fumatore, senza altre comorbidità è seguito da 18 anni presso il Centro per le Dislipidemie Ereditarie della Fondazione Toscana "Gabriele Monasterio" per Ipercolesterolemia Familiare (FH) "Refrattaria" e cardiopatia ischemica precoce, in terapia con acido acetilsalicilico, ipolipemizzanti alle dosi massimamente tollerate (atorvastatina 20 mg/die) e LDL aferesi (Sistema Liposorber®). Per tutto questo periodo, con la terapia sopradescritta, il paziente si è mantenuto stabile sia dal punto di vista metabolico (valori pre LDL aferesi: colesterolo totale 235 mg/dl, HDL 42 mg/dl, trigliceridi 127 mg/dl, LDL 167 mg/dl, Lp "a" 5 mg/dl) che da quello delle condizioni cardiovascolari come evidenziato dalle indagini eseguite nel follow-up (test ergometrico costantemente negativo massimale; eco Doppler vasi cerebrali afferenti con sostanziale stabilità dei reperti ai controlli annuali - a destra: IMT 0.75 mm, stenosi del bulbo del 30%; a sinistra: IMT 1 mm, stenosi del bulbo <30%).

Nonostante il buon andamento clinico, vascolare e metabolico - il migliore ottenibile - nel Luglio 2013 ha riferito episodi di angina da sforzo per cui il paziente è stato sottoposto ad ecocardiogramma da sforzo risultato positivo per ischemia miocardica inducibile e

quindi a studio coronarografico. Questo studio, in assenza di progressione di aterosclerosi coronarica ha evidenziato la presenza di un voluminoso aneurisma fusiforme (diametro massimo 5,8 mm) a carico dell'arteria discendente anteriore, sede di precedente procedura di arteriectomia. Il paziente infatti all'età 48 anni, per angina da sforzo ed evidenza coronarografica di malattia coronarica bivasale, era stato trattato con angioplastica e arteriectomia su discendente anteriore e angioplastica su coronaria destra totalmente occlusa. Procedura complicata da dissecazione subintimale che richiese impianto di stent Palmaz Schatz. A 50 anni, per ripresa di angina, un successivo studio coronarografico aveva mostrato l'occlusione dello stent posizionato a carico della coronaria destra e la pervietà della discendente anteriore.

Da allora il paziente è stato identificato come FH e arruolato al programma specifico di terapia e follow-up insieme a discendenti e collaterali.

Sulla base dell'attuale quadro coronarografico, il paziente è stato sottoposto a rivascularizzazione miocardica mediante confezionamento di singolo by-pass aortocoronarico (LIMA >IVA) senza circolazione extracorporea e con approccio minitoracotomico. Tecnica chirurgica che ha permesso di ottenere una rivascularizzazione efficace, escludere l'aneurisma coronarico e, grazie all'approccio mininvasivo, una più rapida ripresa del paziente. Attualmente il soggetto è asintomatico e non ha più presentato sintomatologia anginoso.

Conclusioni. Il caso qui descritto è riportato a supporto delle tesi di J.S. Forrester e B. Pitt per sottolineare come nella pratica clinica la gestione principale è della complicità cardiovascolare invece che della malattia di base e la rivascularizzazione è proposta come la terapia di maggior qualità.

L'FH, tra le più frequenti malattie ereditarie autosomiche dominanti, è nella maggior parte dei casi non diagnosticata e curata inappropriatamente.

MODIFICHE DI ASSORBIMENTO E SINTESI DEL COLESTEROLO DOPO CHIRURGIA BARIATRICA

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La chirurgia bariatrica si associa ad un significativo calo ponderale, al miglioramento del metabolismo glucidico e lipidico ed alla riduzione del rischio cardiovascolare che è maggiore in coloro con livelli di insulinemia più elevati nel pre-intervento. È noto che la quota di colesterolo plasmatico deriva dal bilancio fra quota sintetizzata e quella assorbita e che soggetti obesi/insulino-resistenti sono caratterizzati da un fenotipo alto sintetizzatore/basso assorbitore. Scopo del nostro studio è stato quello di valutare (gas cromatografia/spettrometria di massa) latosterolo, desmosterolo, campesterolo e sitosterolo, i primi due rispettivamente markers di sintesi e gli altri di assorbimento, in 42 soggetti obesi (BMI 44 kg/m²) prima e 10 mesi dopo un intervento bariatrico, la "sleeve gastrectomy". In un sottogruppo di questi pazienti è stato anche misurato l'intake di macronutrienti.

Dopo l'intervento si è osservata una riduzione di BMI, area del grasso viscerale (VFA), HOMA-IR (p trend <.0001), trigliceridemia ($p.006$) apolipoproteina B ($p.015$) oltre che di latosterolo, desmosterolo, sitosterolo e campesterolo ($p.<.0001$); non modifiche significa-

tive del C-LDL. Dall'indagine alimentare sono emerse riduzioni significative dell'intake di calorie totali, lipidi, colesterolo, grassi saturi, monoinsaturi e polinsaturi (p trend .002). La riduzione di latosterolo è risultata correlata in maniera significativa con la riduzione di campesterolo (.355, p<.05).

All'indagine multivariata solo la riduzione dell'HOMA-IR è risultata predittore del calo di latosterolo e desmosterolo (beta .653 e .674, p<.005); le altre variabili inserite nel modello, non significative, sono la riduzione di BMI, VFA, campesterolo ed intake di colesterolo e grassi saturi.

La riduzione di campesterolo e sitosterolo dopo sleeve gastrectomy potrebbe essere in parte ascrivibile ad una quota di malassorbimento presente nel post intervento mentre la riduzione di desmosterolo e latosterolo, markers di sintesi del colesterolo, è predetta dal calo di insulino resistenza, parametro centrale nell'inquadramento del rischio cardiovascolare nel grande obeso.

SMALL DENSE LDL PARTICLES AND BODY SHAPE INDEX IN A COHORT OF MEDITERRANEAN WOMEN: FINDINGS FROM PROGETTO ATENA

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Small dense LDL particles (sd-LDL) and Body Shape Index (ABSI), a new risk factor for premature mortality indicating that Waist Circumference is higher than expected for a given height and weight and corresponding to a more central concentration of body volume, were evaluated in a population-based cohort study in women, aged 30-69, living in the metropolitan area of Naples, Southern Italy (Progetto ATENA). Serum cholesterol, HDL-cholesterol, LDL-cholesterol, Triglyceride, Insulin, HOMA, Apo B, hs-CPR and sd-LDL were measured in 390 women, as a part of 5.062 participants of the cohort.

LDL particle separation was performed by Lipoprint System: seven LDL subfractions were obtained and LDL score (% of sd-LDL particles) calculated.

ABSI was calculated according to Krakauer's formula: ABSI (m 11/6 kg²/3).

The association between sd-LDL and ABSI was evaluated taking into account different adjustment models.

Women with elevated levels of ABSI (above the 50th percentile) show the following OR of having LDL score (above the 50th percentile):

1.86, 95% Confidence Interval =1.00-3.44, p=0.048; adjusted for age, Systolic Pressure and Triglycerides

2.26, 95% Confidence Interval =1.25-4.08, p=0.007; adjusted for age, Systolic Pressure and diabetes.

1.93, 95% Confidence Interval =1.04-3.59, p=0.036; adjusted for age and Apo B Systolic Pressure and diabetes.

This finding shows that in this group of women ABSI is associated with elevated LDL score above the 50th percentile (score =1.91) independently of age and different cardiovascular risk factors.

These results are in line with the hypothesis that ABSI may be a markers of visceral abdominal fat compared to peripheral tissue associated to adverse metabolic changes including presence of elevated small dense LDL.

STATINE AD ALTA POTENZA E AUMENTO DEL RISCHIO DI DANNO RENALE ACUTO: EVIDENZE DA UN AMPIO STUDIO OSSERVAZIONALE

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Obiettivo. Valutare l'associazione tra danno renale acuto ed esposizione alle statine ad alta potenza rispetto alle statine a bassa potenza.

Disegno. È stato condotto uno studio caso-controllo innestato in una coorte di 316.449 pazienti nella regione Lombardia, di almeno 40 anni, che avevano ricevuto la prima prescrizione di statine nel periodo 2007-2010. I casi erano 458 pazienti ospedalizzati per insufficienza renale acuta (IRA) entro sei mesi dalla prescrizione iniziale di statina. Sono stati selezionati fino a quattro controlli per ogni caso, appaiati per sesso, età e data della prima prescrizione. È stato utilizzato un modello di regressione logistica per valutare il rischio di IRA associato a statine ad alta potenza rispetto a statine a bassa potenza dispensate all'inizio del trattamento o prima dell'evento.

Risultati. I pazienti a cui erano state inizialmente dispensate statine ad alta potenza avevano più probabilità di essere ricoverati per IRA entro sei mesi dall'inizio del trattamento (OR 0,54; IC 95% 1,25-1,91) rispetto a quelli che avevano iniziato la terapia con statine a bassa potenza. I pazienti a cui erano state dispensate statine ad alta potenza entro tre settimane prima della comparsa dell'esito mostravano un aumento del rischio (OR 1,45; IC 95% 1,04-2,03) rispetto a coloro che non avevano ricevuto statine durante la stessa finestra temporale. Non c'erano evidenze di un effetto delle statine sul rischio di malattia renale acuta entro dodici mesi dall'inizio della terapia, né di danno renale cronico.

Conclusioni. Questo studio conferma le osservazioni precedenti di un aumento del rischio di IRA con statine ad alta potenza rispetto alle statine a bassa potenza.

Considerando l'immenso beneficio delle statine nella prevenzione cardiovascolare, la pratica clinica non necessita di sostanziali modifiche. Tuttavia, il potenziale di nefrotossicità delle statine ad alta potenza dovrebbe essere considerato, soprattutto tra i pazienti ad alto rischio di danno renale acuto.

A NOVEL APOB MUTATION IDENTIFIED BY EXOME SEQUENCING COSEGREGATES WITH STEATOSIS, LIVER CANCER AND HYPOCHOLESTEROLEMIA

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Objective. In familial hypobetalipoproteinemia (FHBL), fatty liver is a characteristic feature, and there are several reports of associated cirrhosis and hepatocarcinoma. We investigated a large kindred in which low-density lipoprotein (LDL) cholesterol, fatty liver and hepatocarcinoma displayed an autosomal dominant pattern of inheritance.

Approach and Results. The proband was a 25 year-old female with low plasma cholesterol and hepatic steatosis. Low plasma levels of total cholesterol and fatty liver were observed in 10 more family members; 1 member was affected by liver cirrhosis and four more subjects died of either hepatocarcinoma or carcinoma on cirrhosis. To identify the causal mutation in this family, we performed exome sequencing in two participants with hypocholesterolemia and fatty liver. Approximately 22,400 single nucleotide variants were identified in each sample. After variant filtering, 300 novel shared variants remained. A nonsense variant, p.K2240X due to an A>T mutation in exon 26 of APOB (c.6718A>T) was identified and this variant was confirmed by Sanger sequencing. The genotypic analysis of 16 family members in total showed that this mutation segregated with the low cholesterol trait. In addition, genotyping of the PNPLA3 p.I148M did not show significant frequency differences between carriers and non-carriers of the c.6718A>T APOB gene mutation.

Conclusions. We used exome sequencing to discover a novel nonsense mutation in exon 26 of APOB (p.K2240X) responsible for low cholesterol and fatty liver in a large kindred. This mutation may also be responsible for cirrhosis and liver cancer in this family.

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

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Familial Combined Hyperlipidemia (FCHL) is a severe inherited hyperlipidemia with a high cardiovascular mortality. Affected individuals have elevated cholesterol or triglyceride concentrations

or both. Such a lipid profile is frequently associated with an unfavourable decrease in high density lipoprotein concentration, an elevated apolipoprotein B and an increased prevalence of atherogenic, small, dense low-density lipoprotein (sd-LDL) subfractions. Family studies are necessary to establish the diagnosis of FCHL in each patient.

Since it is not always possible to get some biochemical data from first-degree relatives, the dosage of small dense LDL can be performed in these cases. LDL particles separation is performed by Lipoprint System. The proportion of sd-LDL particles to the whole LDL area is calculated (LDL score). An LDL score higher than 10.0 mg/dL is related in multivariate analysis to FCHL diagnosis, sensitivity 78% and specificity 89% (Atherosclerosis 2009), in addition LDL score is a marker of early carotid atherosclerosis (Chem Chem Acta 2013). In our dataset were screened 173 patients with possible FCHL but without biochemical data from first-degree relatives; 88 patients (50.9%) had LDL score >10 mg/dL and FCHL diagnosis of probable FCHL was done.

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

ASSOCIAZIONE TRA I LIVELLI DI VITAMINA D E PARATORMONE CON LA PULSE WAVE VELOCITY CAROTIDEA IN DONNE POSTMENOPAUSALI IPERTESE

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Introduzione. Le malattie cardiovascolari rappresentano ancora oggi la principale causa di morte nella popolazione anziana. La Stiffness arteriosa o pulse wave velocity (PWV) è ormai riconosciuta come fattore di rischio indipendente di eventi coronarici ed extracoronarici nei pazienti ipertesi. Recentemente anche l'ipovitaminosi D sta emergendo come fattore prognostico negativo per l'ipertensione arteriosa e le cardiomiopatie.

Scopo. Valutare le correlazioni tra la Stiffness arteriosa carotidea ed i livelli circolanti di vitamina D (25-OHD) e paratormone (PTH) nelle donne ipertese in postmenopausa.

Materiali e Metodi. Abbiamo studiato 75 donne ipertese di età superiore ai 60 anni (67,3±7,8) afferite consecutivamente all'Ambulatorio per la Diagnosi e Cura dell'Ipertensione Arteriosa. Le pazienti precedentemente trattate con vitamina D o farmaci per l'osteoporosi sono state escluse. In tutte le pazienti è stato effettuato un prelievo di sangue a digiuno per la determinazione di 25-OHD, PTH, calcemia, fosforemia, creatinina e assetto lipidico. Inoltre è stato effettuato un esame ultrasonografico dei vasi epiaortici mediante Ecografo Esaote MyLab 60 con sonda lineare B-mode da 7.5 MHz, rilevando lo spessore medio-intimale (IMT) e lo Stiffness (PWV) mediante l'elaborazione del software integrato RFQAS.

Risultati. Dopo aggiustamento per l'età PWV è risultata positivamente correlata con la PAS (r=0.32, p<0.01) e il PTH (r=0.35, p<0.001) e inversamente correlata con 25-OH D (r=-0.40; p<0.01). Utilizzando un modello di regressione multivariata abbiamo riscontrato che i livelli di 25-OHD sono un predittore indipendente della PWV carotidea.

Conclusioni. Nelle donne ipertese ultrasessantenni i livelli di 25-OHD sono predittori indipendenti della PWV carotidea. Ulteriori studi longitudinali sono necessari per definire la reale utilità del dosaggio della vitamina D nelle donne ipertese.

IL COLESTEROLO NON-HDL NELL'IPERPARATIROIDISMO PRIMITIVO

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Introduzione. In recenti meta-analisi di trials di trattamento con statine la relazione tra livelli di colesterolo non-HDL (CnonHDL) e rischio di un evento cardiovascolare maggiore sembrerebbe essere più importante rispetto a quella con i livelli di colesterolo LDL (LDL-C) e di apolipoproteina B (ApoB). L'iperparatiroidismo primitivo (pHPT) è noto essere associato ad ipertensione arteriosa, ad incremento della frequenza di intolleranza glicidica e diabete mellito, alterazioni che potrebbero conferire un incremento del rischio cardiovascolare di questi pazienti. Il CnonHDL nell'iperparatiroidismo primitivo non è stato ad oggi studiato ne' sono note eventuali sue associazioni con altri fattori di rischio cardiovascolare.

Soggetti e Metodi. Abbiamo studiato 177 pazienti consecutivi affetti da pHPT (media \pm S.D.: età =59.6 \pm 13.3 aa., M/F =39/138, PTH =232.5 \pm 216.8 ng/l; Calcio =11.3 \pm 1.11 mg/dl). In tutti i pazienti abbiamo misurato il BMI, i livelli pressori, dosato l'assetto lipidico, l'apolipoproteina B, la glicemia basale e l'insulinemia basale (e calcolato l'indice HOMA-IR).

Risultati. Il valore medio di CnonHDL è risultato pari a 152.3 \pm 43.8 mg/dl. Il CnonHDL è risultato ottimamente correlato con i livelli di ApoB (R=0.82, p<0.00001). Abbiamo inoltre osservato correlazioni statisticamente significative anche tra i livelli di CnonHDL ed il BMI (R=0.18, p=0.02), l'età (R=0.23, p=0.017), i livelli di pressione sistolica (R=0.26, p=0.0007), la glicemia (R=0.19, p=0.014), l'insulinemia basale (R=0.25, p=0.014), l'indice HOMA-IR (R=0.26, p=0.012).

Conclusioni. In pazienti affetti da iperparatiroidismo primitivo il valore di colesterolemia non-HDL è strettamente correlato al valore di Apolipoproteina B ed è anche associato ad altri fattori di rischio cardiovascolare. Poiché è una misura semplice, non richiede alcun esame aggiuntivo e le linee guida iniziano a proporre valori target per il trattamento, una valutazione di CnonHDL potrebbe essere di utilità clinica per la valutazione del rischio cardiovascolare di questi pazienti.

PLASMA PROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9) AND PLASMA LIPIDS IN A FREE LIVING POPULATION: RESULTS FROM THE PLIC STUDY

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Background. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a protein convertase expressed in the liver, where it mediates the degradation of hepatic Low density lipoprotein receptor (LDLR), thus inhibiting the clearance of LDL cholesterol (LDL-C).

Objective and Methods. In this study, we determined plasma PCSK9 levels and distribution in the Italian population enrolled in the PLIC study (Progression of Lesions in the Intima of the Carotid, (n= 1518)). Plasma PCSK9 levels were determined by γ -ELISA (Perkin Elmer) and statistical analysis were performed by SPSS software.

Results. Plasma PCSK9 levels are not normally distributed and vary in a wide range from 20.2 ng/mL to 1495 ng/ml and a mean value of 319 \pm 202 ng/mL. PCSK9 plasma levels are significantly higher in women (n=906; 328 \pm 204 ng/mL) than in men (n=612; 305 \pm 198 ng/mL) and in subjects (n=369) under lipid lowering drugs treatment (404.7 \pm 236 ng/mL and 446 \pm 272 ng/mL for statins and fibrates respectively) compared to non-treated subjects (n=1071; 288 \pm 177 ng/mL). Univariate analysis performed excluding subjects under statins and fibrates treatment shows that PCSK9 plasma levels are positively correlated to lipid parameters such as LDL-C (ρ =0.062, p=0.042), ApoB (ρ =0.138, p<0.001), total cholesterol (ρ =0.130, p<0.001), HDL cholesterol (ρ =0.140, p<0.001), plasma triglycerides (ρ =0.111, p<0.001) and Apo-A1 (ρ =0.142, p<0.001). PCSK9 levels positively correlate to the right and left maximum carotid Intima media thickness (maxcIMTdx ρ =0.056, p =0.034 and maxcIMTsx ρ =0.081, p<0.01) but not to the mean cIMT (cIMT ρ =0.026, p=0.331).

Conclusion. In a free living Italian population PCSK9 plasma levels are associated with several lipid parameters such as LDL-C, triglycerides and HDL-C and positively correlate to maximum value of cIMT.

ABDOMINAL VISCERAL FAT MEASUREMENT USING DUAL-ENERGY X-RAY IS ASSOCIATED WITH DETERMINANTS OF METABOLIC SYNDROME

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Background. Obesity is tightly associated with increased cardiovascular risk (CVR). Aim of our study is to examine the association between cardiometabolic risk factors, vascular damage and indexes of adiposity, such as percentage of fat mass (%FM), android/gynoid ratio (A/G) and visceral adipose tissue (VAT) using a dual-energy X-ray absorptiometry (DXA) based approach.

Design and Methods. Analysis of cardiometabolic indicators was conducted on a sample of 939 subjects (541 females and 398 males; average age, 56 years; average BMI, 26 kg/m²) who underwent a total body DXA scan to obtain % FM and A/G. A more detailed measurement of VAT was computed automatically over the DXA android region using CoreScan (GE Healthcare). Common, bulb and internal Carotid Intima-Media Thickness (C-IMT) were measured and presence of plaques were detected through ultrasound.

Results. A/G was positively associated with age (ρ =0.083, p=0.042), C-IMT (ρ =0.174, p<0.001) and it was higher in subjects with plaques in all carotid sections. In contrary % FM was not associated with any of them.

In a more detailed analysis, a sex-specific, age-adjusted multivariable regression model showed that DXA VAT was significantly associated with increased odds of hypertension, impaired fasting glucose, metabolic syndrome, and type 2 diabetes (P<0.001). After

additional model adjustment for BMI and waist circumference, the odds ratio (per SD change in VAT) for type 2 diabetes was 2.07 for women and 2.25 for men. Similarly, the odds ratio for metabolic syndrome for women was 3.46 and for men was 1.75.

Conclusions. DXA provides sturdy information on indexes of CVR in the general population, in particular with the additive data of VAT. This association remains independent from age, BMI, and waist circumference. DXA VAT may provide a more useful device for clinical practice.

THE RELATION BETWEEN MICROALBUMINURIA AND CAROTID INTIMA MEDIA THICKNESS IN TYPE 2 DIABETES

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Background. Vascular endothelium dysfunction is associated with several pathophysiological conditions, including type 2 diabetes (T2DM).

Microalbuminuria has been considered a marker of renal impairment and a predictor of diabetic nephropathy. Moreover, biochemical parameters of endothelial dysfunction and chronic inflammation have been shown to be associated with microalbuminuria. These findings may support the hypothesis that the presence of microalbuminuria reflects a generalized vascular damage. In fact in prospective and epidemiological studies, microalbuminuria has been shown to be independent predictor of cardiovascular events in diabetic patients. Carotid artery intima media thickness (CIMT) is a marker of subclinical atherosclerosis, endothelial damage and a predictor of future cardiovascular events. At present, the relation between microalbuminuria and increased CIMT has been studied with conflicting results. The aim of our study was to analyze the relation between micro-macroalbuminuria and CIMT in an Italian population of T2DM subjects.

Materials and Methods. Two hundred forty two T2DM Caucasian adults were consecutively recruited at the Cardiovascular Prevention Unit of Policlinico Umberto I (Rome, Italy). Anthropometric parameters were measured in all patients. Fasting plasma glucose, serum triglycerides and HDL-cholesterol levels were measured with commercially available enzymatic kits; LDL-cholesterol was calculated using Friedewald formula. Albuminuria was measured on a spot sample of morning urine in 236 patients by an immunoturbidimetric assay. Carotid ultrasound was performed by the same operator blinded to the study protocol using a 7.5 to 10.0 MHz linear transducer (Esaote). The highest common CIMT values were manually recorded. Mean CIMT values were calculated as the mean for the right and left measurements. In a subgroup of 118 subjects CIMT was measured automatically by a specific software (Quality Intima Media Thickness, QIMT, Esaote), which calculates the average of different millimetric measurements on the common carotid artery (QIMT). We compared CIMT and QIMT between normoalbuminuric (NA) and micro-macroalbuminuric (MA) subjects. Parametric variables are expressed as mean \pm SD, while non parametric variables as median (min-max). Age resulted predictor of both CIMT and QIMT. Generalized linear model was used for the multivariate analysis. A $p < 0.05$ was considered statistically significant.

Results. Of the 242 diabetics, 68.5% were males and 31.5% females.

Mean age was 57.9 (± 9.7) years. Mean BMI was 30.6 (± 5.5) kg/m². A significant difference was observed between the two groups (NA=196, MA=46) in gender (NA=126 males, 70 females; MA=38 males, 8 females; $p=0.023$), BMI (NA=30.2 \pm 5.3 kg/m²; MA=32.6 \pm 6 kg/m²; $p=0.019$), glycemic [NA=124 (68 \pm 323) mg/dl; MA=134 (74 \pm 326) mg/dl; $p=0.002$] and triglycerides [NA=117 (41 \pm 577) mg/dl; MA=167 (58 \pm 477) mg/dl; $p < 0.001$] levels.

CIMT values did not differ between NA [0.85 (0.5 \pm 1.85) mm] and MA [0.96 (0.6 \pm 1.75) mm] ($p=0.112$). Conversely, a significant higher value of QIMT was observed in MA individuals (0.85 \pm 0.189 mm) as compared to NA (0.74 \pm 0.14 mm; adjusted $p=0.01$).

Conclusion. Albuminuric patients have a significantly greater QIMT compared to normoalbuminuric patients. This finding may suggest a potential relation between microalbuminuria and CIMT as markers of endothelial dysfunction in type 2 diabetes.

EFFECTS OF A 12-MONTH SUPERVISED EXERCISE PROGRAM ON CARDIORESPIRATORY FITNESS AND METABOLIC PROFILE IN TYPE 2 DIABETIC PATIENTS

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Aim. The aim of our study was to evaluate the efficacy of physical exercise on functional parameters in patients with (T2DM) and on oxidative stress markers, and to prove the role of cardiopulmonary exercise testing (CPX) in the management of the diabetic patient

Methods. We selected 20 male patients with T2DM and metabolic syndrome phenotype, without diabetes-specific complications. They were randomly divided into an intervention group, which followed a supervised physical activity in a hospital-based setting, and into a sedentary control group. The exercise protocol included both aerobic and resistance training, performed for 12 months. Patients underwent medical examination, biochemical investigation, oxidative stress markers dosage and maximal CPX. Oxidative stress markers (1-palmitoyl-2-[5-oxovaleroyl]-sn-glycerol-3-phosphorylcholine [POVPC]; 1-palmitoyl-2-glutaryl-sn-glycerol-3-phosphorylcholine [PGPC]) were measured in plasma and in peripheral blood mononuclear Cells (PBMC). All investigations were carried out at time zero and after 12 months.

Results. In the investigation group we observed a significant increase ($p < 0.05$) in the following parameters: maximum oxygen consumption (+14.4%), anaerobic threshold (+23.4%) and maximum workload (+13.3%). After 12 months, the control group showed a maximum oxygen consumption, a maximum workload and an anaerobic threshold significantly lower than the intervention group. Furthermore, in the intervention group we observed a significant improvement in several metabolic parameters: waist circumference (-1.4%), total cholesterol (-14.6%), LDL-cholesterol (-20.2%), fasting insulinemia (-48.5%), HOMA-IR (-52.5%). The intervention group obtained a significant decrease of plasma levels of POVPC and PGPC at T12 (-27.9% and -31.6%, respectively, without achieving statistical significance in PBMC).

Conclusions. This study confirms the theory that subjects with

T2DM and overweight have a low physical conditioning compared to healthy subjects. The CPX allowed to customize the exercise prescription, that was effective in improving cardiorespiratory fitness, the metabolic asset and the oxidative status.

L'INFIAMMAZIONE DI BASSO GRADO PARTECIPA ALL'AUMENTATO TONO VASOCOSTRITTORE ENDOGENO DI ENDOTELINA-1 NELLE PICCOLE ARTERIE DI PAZIENTI OBESI

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Nel microcircolo periferico di pazienti obesi (Ob) è dimostrato un aumento del tono vasocostrittore (VC) della Endotelina (ET)-1 endogena ed una riduzione della vasodilatazione (VD) mediata dall'Ossido Nitrico (NO). In questo studio abbiamo valutato se il TNF-alfa, localizzato nella parete vascolare e nel tessuto adiposo perivascolare (PVAT), contribuisca alla VC ET-1-dipendente, e se questa azione dipenda dal rilascio tonico di NO, nelle arteriole di resistenza isolate di Ob e controlli (Ctrl). Sono stati studiati 16 Ob (BMI: 44.5±4.7) e 14 Ctrl (BMI: 25.6±4.0), sottoposti ad intervento di chirurgia laparoscopica.

Le arteriole, ottenute da biopsia, sono state studiate con tecnica micromiografica. L'ET-1 endogena è stata valutata mediante risposta al BQ-123, antagonista dei recettori ETA. Il TNF-alfa e l'NO sono stati valutati con Infliximab (IFX), l'anticorpo anti-TNF-alfa, e L-NAME. L'espressione di TNF-alfa, ET-1 e dei recettori ETA è stata valutata nella parete vascolare e nel PVAT. Nei Ctrl, la VC a L-NAME (15.2±0.8%) non era modificata da IFX (15.1±0.9%). Gli Ob mostravano una minore VC a L-NAME (6.0±0.7; P<0.01 vs Ctrl), potenziata (P<0.01) da IFX (16.0±1.0%). Negli Ob, la VD al BQ-123 (51.0±1.5%) era ridotta (P<0.01) da IFX (32.0±1.6%) e resistente a L-NAME (49.0±2.4%). IFX ripristinava l'effetto inibitore di L-NAME sulla VD al BQ-123 (21.0±3.0%; P<0.01 vs BQ-123+IFX). Nei Ctrl, la VD al BQ-123 era ridotta (26.0±1.3%; P<0.01 vs Ob) e immutata da IFX (24.8±1.1%). L-NAME riduceva la risposta al BQ-123 (13.0±0.6%; P<0.01), indipendentemente dalla presenza di IFX (12.1±0.9%). Gli Ob mostravano una overespressione di TNF-alfa nella media (24.9±19.6 vs 2.8±2.5 AU, p<0.001) e nel PVAT (2.9±1.8 vs 1.2±0.7, p<0.005), di ET-1 (45.8±10.3 vs 24.3±15.0, p<0.01) e dei recettori ETA (69.4±6.0 vs 9.6±2.8, p<0.001). Le arteriole di resistenza Ob mostrano un'aumentata VC ETA-dipendente e una ridotta VD NO-mediata. Il TNF-alfa contribuisce all'aumentata VC di ET-1 attraverso la compromissione del rilascio di NO.

IN VIVO ACUTE SYSTEMIC INFLAMMATION, INDEPENDENTLY ON THE ETIOLOGY, AFFECTS HDL CHOLESTEROL EFFLUX CAPACITY

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One of the primary antiatherogenic properties of HDL is its role in promoting reverse cholesterol transport (RCT), a process whereby excess cholesterol is removed from peripheral tissues and transported to the liver for excretion. The capacity of serum HDL to promote efflux of cholesterol from cells (cholesterol efflux capacity, CEC) represents an index of HDL functionality and its evaluation serves to estimate the efficiency of the entire process in humans. It has been suggested that HDL functionality changes may contribute to cardiovascular disease (CVD) protection. Inflammation is a central feature during all stages of atherosclerotic plaque formation with cytokines and chemokines orchestrating the influx of immune cells in disease vessels. Indeed, several systemic inflammatory diseases have been associated with an increased cardiovascular risk. The objective of this study was to analyze whether acute systemic inflammatory disease (sepsis) may affect the capacity of HDL to promote cholesterol efflux (CEC) through the main pathways (aqueous diffusion, AD, the scavenger receptor-BI, SR-BI and the ATP binding cassette transporters A1, ABCA1 and G1, ABCG1). Methods: HDL from 24 patients with sepsis of various etiology and 25 control subjects were tested for their cholesterol efflux capacity (CEC) via the four main pathways by using in vitro cell-based assays. HDL were isolated by precipitating the apoB-containing lipoproteins with PEG.

Results. Patient with sepsis displayed a significant increase in the inflammatory markers such the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), together with a reduction in plasma lipid levels. HDL subclass distribution analysis showed a reduction in medium sized HDL and a trend toward an increase in large HDL in patients with sepsis; the small pre-beta HDL were unchanged in sepsis patients compared to control subjects. Aqueous diffusion (AD)-, SR-BI-, ABCG1-mediated CEC were markedly reduced in all patients with sepsis compared to control subjects (percent reduction in efflux were -28.6%, -6.9% and -24.8%, respectively; p<0.001). ABCA1-mediated CEC remained unchanged between patients and controls (mean percentage efflux ± SEM were 2.82±0.28% compared to 2.84±0.329%).

Conclusion. Subjects with acute systemic inflammation, irrespectively of the etiology of the syndrome, showed impairment of HDL AD-, SR-BI- and ABCG1-mediated CEC; such impairment appear to be the result of structural HDL changes and contribute to explain the accelerated atherosclerosis in these patients.

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