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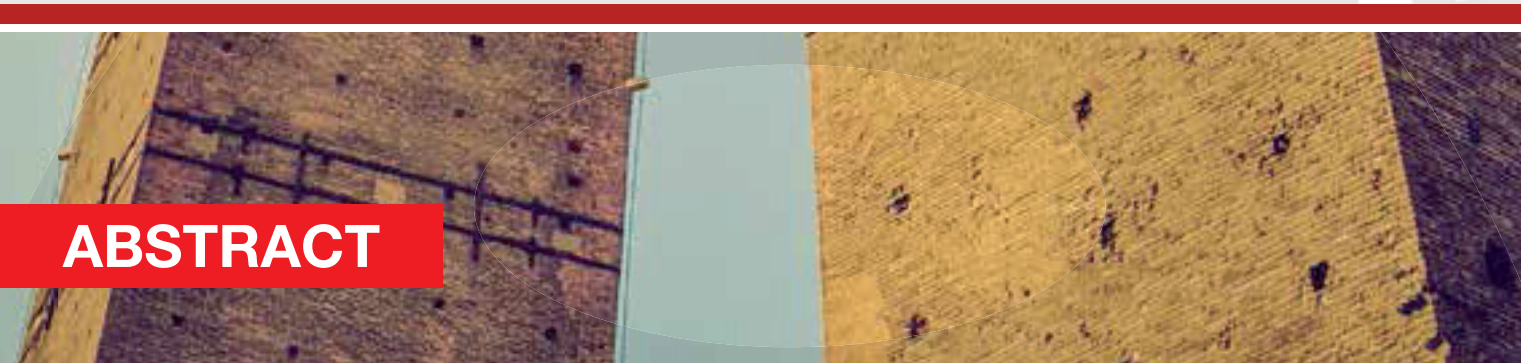

ABSTRACT

IPERLIPEMIA COMBINATA FAMILIARE IN PEDIATRIA: CARATTERIZZAZIONE BIOCHIMICA IN ETÀ PEDIATRICA

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L'Iperlipemia Famigliare Combinata (FCH), patologia ereditaria pro-aterogena del metabolismo lipoproteico, è manifesta nell'adulto ma di dubbia espressione in pediatria. L'incremento di colesterolo totale (CT), trigliceridi (TG) e Apolipoproteina B (ApoB), variabilità fenotipica intra-individuale/intra-famigliare ed eventi cardiovascolari precoci (pCVD) rappresentano parametri diagnostici comuni, in assenza di marker specifico. L'alterata distribuzione del colesterolo nelle frazioni lipoproteiche, analizzata mediante ultracentrifugazione in gradiente di densità (DGUC), e la caratterizzazione di lipoproteine a bassa densità piccole e dense (sdLDL) contribuiscono alla diagnosi nell'adulto. In età pediatrica è stato dimostrato che l'eccesso ponderale può influenzare il fenotipo biochimico ma a differenza dell'adulto mancano dati relativi all'analisi della distribuzione del colesterolo nelle frazioni lipoproteiche.
Obiettivo dello studio. Caratterizzazione fenotipica di soggetti pediatrici FCH e analisi della distribuzione del colesterolo nelle frazioni lipoproteiche al fine da valutare le implicazioni relative al rischio cardiovascolare di tali soggetti.
Materiali e Metodi. Pazienti: 61 soggetti 6-16 anni (50 famiglie) FCH diagnosticati sulla base di livelli di CT e TG superiori al 90° percentile per sesso ed età, associati a ipercolesterolemia e/o ipertrigliceridemia in almeno 2 membri della famiglia (uno di primo e uno di secondo grado) con variabilità fenotipica intra-familiare. Esaminati 11 soggetti controllo appaiati per sesso ed età.
Biochimica: Dosaggio sierico di CT, colesterolo HDL (HDL-C) e TG con metodo enzimatico-colorimetrico e di ApoB, ApoA-1 con metodo immunoturbidimetrico. Determinazione della distribuzione del colesterolo nelle varie frazioni lipoproteiche è stata effettuata mediante DGUC.
Statistica: Valutazione dei risultati applicando Test del Chi Quadrato e Test esatto di Fisher per le variabili categoriche; Test t per campioni indipendenti e il test ANOVA a 2 vie per le variabili quantitative. L'analisi di correlazione di Pearson è stata applicata per valutare l'associazione tra età, BMI e livelli sierici delle lipoproteine.
Risultati. L'espressione di FCH è risultata eterogenea con prevalenza del fenotipo IIb (ipercolesterolemia e ipertrigliceridemia), rispetto al fenotipo IIa (ipercolesterolemia isolata) e al fenotipo IV (ipertrigliceridemia) sia nei probandi pediatrici che nei famigliari affetti. È stata osservata la presenza di pCVD nel 40% delle famiglie. I soggetti FCH presentano livelli significativamente maggiori di CT, TG, LDL-C e ApoB, ApoB/ApoA1 rispetto ai controlli mentre non si riscontrano differenze significative in relazione ai livelli di HDL-C e ApoA-1. Nei soggetti FCH si osserva una maggiore prevalenza di sovrappeso/obesità rispetto ai controlli con una correlazione significativa tra BMI e livelli di TG. La distribuzione del colesterolo evidenzia un maggior contenuto di colesterolo nei soggetti FCH rispetto ai controlli nelle frazioni HDL1-2, LDL12-18, IDL 19-28 e VLDL. Il confronto della distribuzione in relazione al fenotipo evidenzia maggior contenuto di colesterolo nelle frazioni HDL, LDL (frazioni di LDL più larghe e meno dense) nel fenotipo IIa, e significativamente maggiore nelle VLDL in soggetti con fenotipo IIb. Soggetti con fenotipo IV presentano minor contenuto di colesterolo nelle HDL, LDL e parte delle frazioni IDL. Confrontando soggetti normopeso con sovrappeso/obesi il picco di densità delle LDL è spostato verso le frazioni più piccole e dense nei secondi.
Conclusioni. Questi risultati evidenziano che FCH è un disordine pro-aterogeno del metabolismo lipidico per cui è possibile e opportuno eseguire la diagnosi in età pediatrica. Ai parametri tradizionali, e oltre al dosaggio di ApoB, l'analisi quantitativa della distribuzione del colesterolo mediante DGUC nella presente casistica ha evidenziato che in età pediatrica il profilo della FCH risulta correlato all'aumento del contenuto totale di colesterolo distribuito nelle LDL più larghe (in particolare nei soggetti con ipercolesterolemia isolata), IDL e VLDL (in particolare nei soggetti con fenotipo combinato). La condizione di sovrappeso/obesità aggrava il profilo aterogeno pertanto l'ottimizzazione dello stile di vita risulta fondamentale nei soggetti FCH in età pediatrica per ridurre il loro rischio cardiovascolare.

EFFETTI ANTIATEROSCLEROTICI DI UNA NUOVA COMBINAZIONE DI BERBERINA, MONACOLINA K E MORUS ALBA: MIGLIORAMENTO DELLA FUNZIONALITÀ DELLE HDL

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Scopo. La capacità di promuovere l'efflusso del colesterolo (CEC) rappresenta la capacità delle HDL di rimuovere il colesterolo in eccesso dai macrofagi ed è stata recentemente correlata all'incidenza di eventi cardiovascolari, indipendentemente livelli plasmatici di colesterolo HDL (HDL-C). Abbiamo valutato l'effetto di una combinazione nutraceutica a base di Berberina (531,25 mg), riso rosso fermentato (220 mg; 3,3 mg di monacolina K) e Morus Alba (200 mg) sulla CEC delle HDL. Inoltre, abbiamo misurato l'effetto della capacità del siero di accumulare colesterolo nel macrofago (cholesterol loading capacity CLC), un indice della capacità complessiva delle lipoproteine sieriche di caricare macrofagi con colesterolo e di indurre la formazione delle foam cells.
Metodi. 9 volontari sani sono stati trattati per 4 settimane con la combinazione nutraceutica descritta sopra. La CEC delle HDL attraverso i singoli meccanismi di efflusso è stata valutata con un saggio radioisotopico. La CLC del siero è stata misurata con un saggio fluorimetrico.
Risultati. Il trattamento con la combinazione nutraceutica ha ridotto significativamente il colesterolo totale, LDL (-14% e -17%, $p<0,01$ <0,05, rispettivamente) e HDL (-11,7%; $p<0,01$). Il trattamento non ha influenzato la CEC tramite ABCA1, ma ha significativamente aumentato la CEC mediata da SR-BI e ABCG1 (+27% e +13%, rispettivamente; $p<0.05$). Infine, la combinazione nutraceutica ha ridotto la CLC del siero (-23,2%; $p<0,05$).
Conclusioni. Nonostante l'effetto sui livelli plasmatici di HDL-C, il trattamento con la combinazione nutraceutica ha migliorato la principale funzione antiaterogena delle HDL. In particolare, il trat-

tamento ha selettivamente incrementato la CEC mediata da SR-BI e ABCG1, suggerendo una ridistribuzione delle HDL verso particelle specifici per questi trasportatori. Sia il miglioramento della funzionalità delle HDL che l'abbassamento dei livelli plasmatici di colesterolo LDL possono contribuire alla riduzione del potenziale pro-aterogeno del siero (CLC) osservato dopo trattamento.

RELAZIONE TRA HDL-C E VASCULOPATIA PERIFERICA IN SOGGETTI AFFETTI DA DIABETE MELLITO TIPO 2 DI NEODIAGNOSI E/O BREVE DURATA DI MALATTIA

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Introduzione e Obiettivi. La relazione tra basso HDL-C e vasculopatia periferica nei soggetti affetti da diabete mellito di tipo 2 (DMT2) sembra essere presente in pazienti con lunga durata di malattia e scompenso glicemico. Obiettivo del presente studio è stato valutare tale relazione in un gruppo di pazienti affetti da DMT2 con breve durata di malattia e in buon compenso glicemico.
Materiali e Metodi. È stato condotto uno studio osservazionale su 541 soggetti affetti da DMT2, arruolati consecutivamente presso il Centro di Diabetologia e prevenzione cardiovascolare del Policlinico Umberto I di Roma. La diagnosi di vasculopatia periferica è stata definita dal valore dell'indice caviglia- braccio ≤0,9. I pazienti sono stati divisi in due gruppi, "basso HDL-C" e "alto HDL-C" in base ai criteri classificativi delle linee guida NCEP-ATP III e, secondariamente, suddivisi in ulteriori due cut off HDL-C <40 mg/dl o >60 mg/dl, rispettivamente. È stato considerato significativo un valore di p<0,05.
Risultati. I soggetti arruolati erano prevalentemente maschi, sovrappeso, con breve durata di malattia (mediana 3 anni) e in buon compenso glicemico (mediana HbA1c 7%). Un terzo dei soggetti era fumatore. Nel gruppo di soggetti con basso HDL-C secondo i criteri NCEP-ATP III, la prevalenza e il rischio relativo di vasculopatia periferica erano maggiori rispetto ai soggetti con valori di HDL normali (17,3% vs 10,7%; p=0,035) (OR 1,75; IC 95%: 1,035-2,955; p=0,037). Tale relazione perdeva di significatività all'analisi multivariata, correggendo per la variabile fumo (OR 1,57; IC 95%: 0,913-2,712; p=0,102). I dati sulla maggior prevalenza e rischio relativo si confermavano nel confronto tra i gruppi con HDL-C <40 mg/dl e >60 mg/dl. In particolare, all'analisi multivariata, i soggetti con HDL-C <40 mg/dl presentavano un RR di circa 2.4 volte maggiore rispetto ai soggetti con HDL-C >60 mg/dl di vasculopatia periferica, dato al limite della significatività statistica (OR 2,37; IC 95%: 0,916-6,114; p=0,075).
Conclusioni. Nella popolazione esaminata, i soggetti con basso HDL-C presentano maggior prevalenza e rischio relativo di vasculopatia periferica. Tale relazione sembra in parte mediata dall'effetto del fumo sulla colesterolemia HDL-C. Sono necessari ulteriori studi per confermare il possibile ruolo del basso HDL-C come fattore di rischio precoce per la vasculopatia periferica nel DMT2.

CHEMICAL STRUCTURE HIERARCHICAL CLUSTERING ANALYSIS APPLIED TO LIPIDOMICS USING TWO CUSTOM APPLICATIONS: FRAGCLUST AND TESTCLUST

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Lipidomics analysis is able to measure simultaneously thousands of compounds belonging to few lipid classes. In a single lipid class, compounds differ only by their acyl radicals, ranging between C10:0 (capric) and C24:0 (lignoceric) in biological matrices. Though in some cases single metabolites showed to play a peculiar pathological role, more often many compounds from a single lipid class exert the same biological effect. In this work we present a lipidomics workflow that extract the tandem mass (MS/MS) data from individual files and use them to group compounds in structurally homogeneous clusters by chemical structure hierarchical clustering analysis (CHCA). The case/control peak area ratios of metabolites are then analyzed as clusters. We created two freely available applications to assist the workflow: FragClust that helps to generate the tables to be subjected to CHCA, and TestClust, that performs statistical analysis on clustered data. To test this new method we used the lipidomics data from Alzheimer's Disease (AD) patient's plasma in comparison with healthy controls. Up to date the search for plasma biomarkers in AD has not provided reliable results. The results shows that our workflow was helpful to understand the behavior of whole lipid classes in AD plasma. In general this novel analytical approach could be useful to find lipid pathways related to different pathological conditions.

INTOLLERANZA ALLE STATINE NEI PAZIENTI AFFERENTI AGLI AMBULATORI DEDICATI ALLE DISLIPIDEMIE

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Introduzione. Le statine, farmaci largamente utilizzati, vengono frequentemente sospese per disturbi a carico del sistema muscolo scheletrico.
Scopo. Verificare l'intolleranza alle statine (IS) nei pazienti che afferiscono agli ambulatori dedicati alle dislipidemie.
Metodi. Abbiamo valutato retrospettivamente i dati clinici dei pazienti afferenti agli ambulatori per le dislipidemie nel 2014. Abbiamo raccolto parametri antropometrici, storia familiare/personale di malattia cardiovascolare, terapia ipolipemizzante, profilo

lipidico, transaminasi, CPK. IS è stata definita come sospensione del trattamento per mialgia (senza rialzo CPK), rialzo asintomatico CPK (aumento CPK di tre volte il limite superiore di norma), concomitanza di mialgia-rialzo CPK, aumento asintomatico delle transaminasi (tre volte il limite superiore di norma).
Risultati. Abbiamo raccolto i dati di 217 pazienti (98 M, 119F) età media 55.2±15.3 anni. 57 pazienti (26.2%) presentavano IS (63% mialgia, 1,7% rialzo asintomatico CPK, 24,5% mialgia-rialzo CPK, 8,7% aumento delle transaminasi). Degli intolleranti, 16 (28%) avevano sospeso la terapia. Il colesterolo-LDL negli intolleranti risultava significativamente maggiore rispetto ai tolleranti (133.21±32.19 vs 117.72±38.16 mg/dl, p=0.016). Inoltre, solo l'8,5% degli intolleranti presentava valori di col-LDL <100 mg/dl (vs 34% dei tolleranti); e il 53,2% degli intolleranti presentava colesterolo-LDL >130 mg/dl (vs 32% dei tolleranti): p=0,002 per entrambi i confronti. Nel sottogruppo di pazienti con ipercolesterolemia familiare eterozigote (FH) (92 pazienti, di cui 35 con diagnosi molecolare di mutazione del R-LDL), l'IS riguardava 28 pazienti (30.4%),con sospensione della statine in 11 soggetti (39%), per mialgia (50%), aumento asintomatico di CPK (3,5%), mialgia associata ad aumento di CPK (39%) e aumento asintomatico delle transaminasi (7,1%).
Conclusioni. L'IS è di frequente riscontro nella pratica clinica, riguarda circa 1/4 dei pazienti ed è responsabile della sospensione della terapia nel 28%. Nei pazienti FH l'IS interessa 1/3 dei pazienti con sospensione nel 39%. Gli intolleranti presentano maggiori livelli di colesterolo-LDL e sono meno frequentemente a target.

EPCs BEFORE AND AFTER TREATMENT WITH METFORMIN IN PATIENTS WITH FCHL AND INSULIN RESISTANCE

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Background. Familial combined hyperlipidemia (FCHL) is a common disorder of metabolism, associated to increased cardiovascular (CV) risk; besides the overproduction of very low-density lipoprotein and altered remnants catabolism, a high prevalence of insulin resistance (IR) has been also described in FCHL subjects. IR is known to be associated with low-grade chronic systemic inflammation, and is a strong predictor of CV disease. Endothelial progenitor cells (EPCs) are a heterogeneous population of cells with the ability to differentiate into cell types of different organs and systems, and are vital for the maintenance and repair of the endothelium. We have already evaluated CD34+EPCs in several clinical conditions, characterized by both high-grade and low-grade inflammation, and we found inverse associations between disease severity and cell count. The antidiabetic drug metformin, which improves insulin sensitivity and reduces hepatic glucose production, was reported to increase EPC levels in diabetic patients. We sought to evaluate whether metformin could improve CD34+EPC count in non-diabetic, FCHL/IR patients.
Methods and Results. We evaluated CD34+EPC count in newly diagnosed FCHL/IR patients at baseline (T0) and after six-months of metformin treatment (T1). We enrolled 29 patients (M:F 20:9; age 46±11 years) with no further CV or metabolic risk factor. After metformin treatment, there was a significant reduction in fasting plasma glucose (Δ=-4.45%; p<0.05), plasma insulin (Δ=-18%; p<0.05), HOMA-IR (Δ=-19.9%; p<0.05) and an increase in CD34+EPC count (Δ=69.1%; p<0.05). Moreover, we found an inverse correlation between CD34+EPC count and plasma insulin (r-0.571, p=0.01) and HOMA-IR (r-0.583, p=0.009). Dependence analysis showed an association between ΔHOMA and ΔCD34+ cells (t -2.961, p=0.009).
Conclusion. Our study suggests that in FCHL/IR patients metformin, possibly by improving insulin-sensitivity, may increase CD34+EPC amount, which could positively impact CV risk in these patients.

OxLDL CONTRIBUTION TO ARRHYTHMOGENIC CARDIOMYOPATHY PHENOTYPIC MANIFESTATION

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Arrhythmogenic Cardiomyopathy (ACM) is a genetic disease characterized by fibro-adipose substitution of ventricular myocardium, ventricular tachycardias and sudden cardiac death, mainly in young athletes. Different causative genes have been identified, among which PKP2, but variable penetrance and expressivity suggest the involvement of other genetic, epigenetic or environmental factors.
A cohort of 16 consecutive ACM patients showed slight, but consistent higher total (211.3±8.0 vs 185.0±6.8; p=0.020), LDL cholesterol (133.4±30.8 vs 111.1±20.7; p=0.031) and oxLDL (217.1±88.5 vs 106.9±36.6) levels compared to gender and age-matched healthy controls. We hypothesize that soft dyslipidemias and oxidative stress, possibly induced by exercise, may act as ACM cofactors.
Using Cardiac Mesenchymal Stromal Cells (C-MSC) as in vitro model for ACM, we demonstrated that an oxidized fatty acid, 13HODE, present in oxLDL, promotes lipid accumulation specifically in ACM C-MSC (n=3; 259.8±42.5 vs 135.5±24.4; p=0.019), while the activity of the antioxidant N-acetylcysteine inhibits it (21.3±6.9 vs 105.3±77.3; p=0.007). 13HODE is a known activator of PPARγ, one of the main adipogenesis regulators. Transcriptional activation of PPARγ in ACM cells (n=5; 5.0±1.3 vs 1.4±0.7; p=0.044) was observed in parallel with that of CD36 (116.0±67.8 vs. 1.8±0.5; p=0.042), oxLDL receptor, whose expression is PPARγ-dependent. Although the mouse model of ACM (PKP2-KO) does not accumulate fat cells in the heart, probably for intrinsic protection mechanisms, their C-MSC are predisposed to lipid accumulation in vitro (22.8%±2.5% vs 12.7%±3.1%; p=0.015). By administering to ACM mice (n=5) a high-fat diet (shown to increase the circulating LDL) we observed a fibro-fatty replacement in vivo.
We can therefore assume that a mutation in the genes ACM is necessary but not sufficient for severe manifestation of the disease and that elevated plasma LDL and oxidative stress are cofactors in the pathogenesis. Lipidomics analysis are underway to understand these mechanisms.

STUDIES ON THE MECHANISM OF ACTION OF THE ANTIPROLIFERATIVE EFFECT OF NO-DONORS FUROXANS IN SMOOTH MUSCLE CELLS. A GREEN LIGHT FOR A POSSIBLE INNOVATIVE ANTI-ATHEROSCLEROTIC APPROACH?

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Atherosclerosis is characterized by oxidation, altered NO production and increased smooth muscle cell (SMC) proliferation in vascular intima. In order to find innovative antiatherosclerotic approaches, we synthesized NO-donor molecules (furoxans) able to release aortic rings in a NO-dependent fashion and to inhibit SMC proliferation. Based on the structure of 4-phenyl-1,2,5-oxadiazol-N-oxide (4-phenyl-furoxan), our results demonstrated that the antiproliferative effect is related to the electron-attractor power of the substituent in position 3 of the aromatic ring. Experiments conducted on corresponding nor-derivatives 4-phenyl-1,2,5-oxadiazoles (furazans) showed almost no inhibitory activity. Since our experimental evidence seem to exclude that the antiproliferative properties are mediated by NO through the cGMP- or the polyamines pathway, to exclude a different NO-mediated effect, we evaluated whether this inhibitory effect would still be present after co-treatment with classical NO-donor scavengers (red globules, hemoglobin). These molecules prevent SMC growth inhibition but, unfortunately, their effect seems to be due to the presence of thiol groups (which degrade furoxans) in their backbones, rather than specifically scavenge NO. After having tested other non-classic NO scavengers, we are currently evaluating the effects of PTIO, a NO-scavenger without thiols and results are not yeat available. Since at present we can not exclude that the antiproliferative effect of furoxans could be independent of NO, we are also exploring a more complex and systematic proteomic approach. By 1D- and 2D-gel electrophoresis of cell lysates, imaging and MS analysis, we are trying to identify potential cellular targets of furoxans (enzymes and proteins): hyperexpression, phosphorylation, S-nitrosylation of proteins involved in G1/S phase transition of the cell-cycle may be responsible for their antiproliferative effect. Once the mechanism of action will be understood, these molecules may be utilized as antiproliferative drugs or being hybridated with other pharmacophores to find innovative approaches in the treatment of atherosclerosis.

ANALYSIS OF SERUM AND LIVER LIPID PROFILE OF RATS WITH NAFLD INDUCED BY A METHIONINE AND CHOLINE DEFICIENT DIET AND TREATED WITH EUROSIL85

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The non-alcoholic fatty liver (NAFLD) is a pathology that ranges from steatosis up to steatohepatitis-fibrosis (NASH) and cirrhosis-hepatocarcinoma. Risk factors include obesity, insulin resistance, hyperglycemia and hypertriglyceridemia. Since, following the "two hits" theory, the first injury causes lipid accumulation and the second triggers inflammation and fibrosis, lipids, oxidative stress and inflammation are preminently involved in NAFLD development. Because targeted pharmacological treatment are lacking, new therapies based on antiinflammatory, hypolipidemic, antioxidant and antifibrotic molecules are needed. Among these, Silymarin, a mixture of polyphenols extracted from Silybum Marianum may be a valid opportunity. The increased bioavailability of the pharmaceutical formulation Eurosil85 (Rottapharm/MEDA) prompted us to evaluate its effectiveness in 56 male Wistar rats, divided in 4 groups (14 each) and fed:

- a diet enriched in fats and sucrose, without methionine and choline (MCD);
- MCD diet plus Eurosil85 40- or 200 mg/kg/day;
- MCD diet supplemented with methionine and choline (MCS; control diet) for 1-3 months.

One month of treatment is safe (ALT e GGT unchanged), despite MCD diet does not allow rats to gain weight. Serum cholesterol increases in MCS rats vs MCD, but Eurosil85 200 mg/kg/day reduces it significantly (-30%). While MCS diet enhances serum TG (vs MCD), Eurosil85 does not (vs MCD), but it ameliorates serum total antioxidant power vs MCD rats.
One month of MCD diet (vs MCS) increases liver cholesteryl esters, TG and free fatty acids, while increasing polyunsaturated fatty acid relative content. While phospholipid mass (mainly phosphatidylcholine) is decreased by MCD diet (dietary lack of the precursor choline), Eurosil85 200 mg/kg/die decreases hepatic free cholesterol without altering other lipid parameters. We are currently evaluating hepatic lipids, inflammation, oxidative parameters and fibrosis after three month-treatment to assess whether Eurosil85 is a valid pharmacological approach in the treatment of NAFLD.

VALUTAZIONE DELL'EFFETTO DELLA DIETA MEDITERRANEA IN PAZIENTI IPERTRIGLICERIDEMICI IN RELAZIONE A VARIANTI GENETICHE COMUNI DEL GENE APOA5

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Introduzione e scopo dello studio. L'ipertrigliceridemia (HTG) "moderata" è definita per valori di TG tra 150-885 mg/dl, e incrementa il rischio di coronaropatia aterosclerotica (CAD). Numerosi studi comprovano l'efficacia della dieta mediterranea nel ridurre il rischio cardiovascolare. Le varianti comuni del gene APOA5 sono considerate tra i più forti modulatori della trigliceridemia. Scopo del lavoro è valutare in pazienti affetti da ipertrigliceridemia moderata l'efficacia della dieta mediterranea nel ridurre i parametri antropometrici, lipidici e glicemia e valutare se il profilo lipidico e l'effetto della dieta sono modulati dalle varianti genetiche comuni dell'APOA5: -1131T>C e C56>G (p. Ser19>Trp).
Metodi. Lo studio è stato svolto su 83 pazienti maggiorenni afferenti presso l'ambulatorio delle Dislipidemie dell'IRCCS AOU San Martino IST affetti da HTG moderata. Sono stati valutati al basale e dopo 6 mesi, i parametri antropometrici, il profilo lipidico completo, la glicemia ed è stato effettuato un prelievo di sangue per l'analisi del DNA. Ai pazienti è stata proposta una dieta mediterranea da 2.100 kcal negli uomini e da 1.700 kcal nelle donne.
Risultati. Tutti i parametri valutati (crf vita, BMI, colesterolo to-

tale, LDL, TG, glicemia), sono diminuiti significativamente dopo sei mesi di trattamento (p<0,0001). I livelli di HDL sono aumentati significativamente (p<0,007). Analizzando i sottogruppi in relazione ai polimorfismi genetici si osserva che al basale i trigliceridi sono significativamente più elevati nei portatori della variante Trp rispetto ai non portatori (363±193.6 vs 282,3±105,3; p<0.02); Anche nel caso della variante del promotore, i portatori dell'allele C presentano al basale livelli di trigliceridi più elevati rispetto agli omozigoti TT (350,1±194,1 vs 284,1±103,0; p<0,05). Dopo 6 mesi di dieta mediterranea i livelli di trigliceridi complessivamente si riducono e si perde la significatività statistica delle differenze tra portatori e non portatori di varianti genetiche.
Conclusione. La dieta mediterranea è efficace nel migliorare i parametri lipidici, il BMI la circonferenza vita e la glicemia ma in particolare riduce la trigliceridemia anche in presenza di genotipi sfavorevoli.

ROLE OF PCSK9 (PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9) IN OBESITY AND METABOLIC SYNDROME: BEYOND LDL-R TARGETING

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Objective. PCSK9, a liver-secreted plasma enzyme, primarily regulates the levels of circulating low-density lipoprotein cholesterol (LDL-C) by enhancing the degradation of the hepatic LDL receptor (LDLR). The emerging importance of PCSK9 inhibition for the treatment of hypercholesterolemia warrants investigation of the physiological role of PCSK9, beyond LDL-C lowering. Indeed, PCSK9 targets additional receptors which could play a critical role in triglyceride-rich lipoproteins (TGRL) metabolism, thus potentially affecting metabolic syndrome and obesity.
Methods and Results. Metabolic disfunction was induced in 2-months old PCSK9 KO and WT male mice by feeding them for 20 weeks with a HFD (High Fat Diet) or SFD (Standard Fat Diet). As expected, the deletion of PSCK9 resulted in a significant reduction in plasma cholesterol levels (86,1±2,1 mg/dl vs 123,4±5,2 mg/dl and 51,8±12,3 mg/dl vs 79,8±11,0 mg/dl with HFD and SFD respectively, p<0,05), but not in plasma triglyceride levels. Of note, PCSK9 KO mice accumulated significant more visceral adipose tissue than WT littermates (+50%±17% with HFD, p<0,05) with a trend towards increased weight gain. Further assessement of metabolic dysfunction development showed that PCSK9 deficiency resulted in impaired glucose tolerance compared to control mice (Glucose Tolerance Test - Area Under the Curve +40%±9% with HFD, p<0,05), while the response to insulin was not affected.
Conclusion. Taken together our data indicate that, under impaired metabolic setting, PCSK9 deficiency results in reduced glucose tolerance and in increased visceral adipose tissue accumulation.

DISLIPIDEMIA IN PEDIATRIA E MISURAZIONE DELLO SPESSORE MEDIO INTIMALE

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La valutazione dello spessore medio-intimale, praticata correntemente nell'adulto, è proposta dall'evidenza come marker surrogato di aterosclerosi preclinica recentemente anche in ambito pediatrico.
Obiettivi dello studio. Analisi dell'applicabilità della tecnica in bambini ed adolescenti dislipidemici e confronto tra la predittività della misurazione nei tratti carotideo ed aortico.
La misurazione è effettuata in soggetti tra 5 e 17 anni, 274 con iperlipemia primitiva e 47 controlli, con ecografia B-mode e sonda lineare a multifrequenza 7,5-10MHz.
Sono confrontati gli spessori dei dislipidemici con quelli dei controlli; successivamente sono confrontati i dislipidemici divisi per la relativa diagnosi (Ipercolesterolemia Familiare, Ipercolesterolemia Familiare Combinata ed Ipercolesterolemia ndd). Sono studiate le influenze di sesso, età, BMI, familiarità e profilo lipidico sullo spessore medio-intimale e confrontate le informazioni ottenute dai due distretti vascolari.
I dislipidemici, soprattutto con Ipercolesterolemia familiare, mostrano spessore medio-intimale carotideo ed aortico significativamente aumentati rispetto ai controlli.
Età, BMI e diagnosi di Ipercolesterolemia Familiare risultano predittivi per aumento dello spessore medio-intimale carotideo, mentre età, BMI ed LDL-colesterolo per quello aortico.
Selezionando i pazienti per presenza di familiarità, valori elevati di BMI o alterato profilo lipidico, lo spessore medio-intimale aortico è aumentato significativamente in chi presenta più di un fattore di rischio.
Dividendo il gruppo con Ipercolesterolemia Familiare in terzili di l'LDL-colesterolo, lo spessore medio-intimale aortico è significativamente aumentato nell'ultimo e c'è una progressione significativa dal primo al secondo terzile e dal secondo al terzo.
La misurazione non invasiva dello spessore medio-intimale è quindi affidabile nel bambino dislipidemico.
Si conferma l'applicabilità nel tratto carotideo e si aggiunge che l'esposizione a fattori di rischio cardiovascolare sia associata ad una maggiore progressione di quello aortico.
Elevati livelli di LDL-colesterolo espongono ad un'aterosclerosi accelerato e si individua un sottogruppo con Ipercolesterolemia Familiare ad alto rischio di aumento dello spessore medio-intimale aortico, che necessita un follow-up più aggressivo.

LEUKOCYTE TELOMERE LENGTH, GENETICALLY DETERMINED, IS CAUSALLY ASSOCIATED WITH THE PROGRESSION OF CAROTID INTIMA-MEDIA THICKNESS

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Introduction. Leukocyte Telomere Length (LTL) is an index of telomere functionality and of cellular replicative capacity. Progressively with aging, LTL shortening (TS) occurs; however, and increased TS has been reported in the context of several disease, like cancer and diabetes. Aim of our work was to:
1) evaluate if LTL and TS were associated with carotid Intima-Media Thickness (c-IMT), marker of subclinical atherosclerosis and cardiovascular prognosis;
2) determine if LTL and TS are determined by genetic signatures;
3) evaluate the association of this genetic profiles with c-IMT progression.

Materials and Methods. Therefore we measured basal LTL on a representative cohort of the general population (Studio PLIC); six years later the measurement was replicated on 768 subjects. Of these, clinical, biochemical, anthropometric and pharmacological data were collected. c-IMT was measured by ultrasound at both visit to evaluate its progression. SNPs on seven GWAS hit loci were available (five of them are on candidate genes encoding for components of the telomerase complex: TERC, TERT, NAF1, OBFC1 and RTEL1). Genetic risk score was then calculated for each subject.

Results. Median basal LTL was 5.75 kbp [3.59-8.26] and 4.61 kbp [3.64-5.53] after 6 years. Median TS was -956 bp [IQR -3.536-1.185]. LTL was inversely associated with TS (rho=-0.919, p<0.001). LTL and TS were associated with different cardio-metabolic parameters but not with pharmacological therapy. Adjusting for risk factors, TS was associated with faster c-IMT progression (beta=1.48; p=0.05). TS varied as a function of the genetic risk score (beta =-0.090, p=0.041), which was, in turn, correlated to faster c-IMT progression.

Conclusions. In conclusion, LTL and TS, hereditary traits, were associated with c-IMT progression. Genetic analysis suggests TS to be causally associated with the progression of subclinical atherosclerosis.

IMPAIRMENT OF GLP-1 EFFECTS IN PLATELETS FROM SUBJECTS AFFECTED BY TYPE 2 DIABETES MELLITUS

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Background and Aims. Glucagon-like peptide 1 (GLP-1) exerts crucial metabolic effects, thus justifying the GLP-1-based therapies as treatment options for patients affected by T2DM, and shows antiatherogenic properties by influencing cardiovascular system. The bioactive GLP-1 exists principally as GLP-1 (7-36), a peptide rapidly degraded by specific enzymes to GLP-1 (9-36). Platelets play a pivotal role not only in haemostasis, but also in vascular inflammation and atherothrombosis, and T2DM has been considered to be a prothrombotic status. Aim of the study was to investigate the GLP-1 influence on platelet function in T2DM.

Methods. In platelets from 20 T2DM patients (M/F: 9/11; age 56.1±1.9 years; BMI: 32.6±1.1 kg/m^2; HbA1C:8.0±0.2%) and 12 healthy control subjects (M/F:6/6; age: 48.2±3.4 years; BMI: 24.0±0.7 kg/m^2) we evaluated the ability of GLP-1 (7-36), GLP-1 (9-36) and Liraglutide (100 nmol/l) to interfere with:
1) the inhibitory action of nitric oxide (NO) by measuring the anti-aggregating effects of the NO donor Na-nitroprusside (SNP) (5 micromol/l) (Born's method);
2) the phosphorylation levels of AKT (pAKT), a downstream molecule of PI-3 kinase pathway activation, ERK-1/2 (pERK-1/2), which mirrors activation of MAP-kinase pathway (Western Blot) and ROS synthesis (DCF-DA fluorescence assay) stimulated by the agonists ADP (10 micromol/l), Collagen (4 mg/L) and Na-Arachidonate (NaA) (100 micromol/l).

Results. GLP-1 metabolites increased the antiaggregating effects of SNP and decreased the agonist-induced activation of PI-3K/AKT, MAPK/ERK-2 and ROS production but these effects were lower in T2DM than in healthy subjects. In particular, in the presence of GLP-1 (7-36): the percent increases of the antiaggregating effects of SNP were 4.0±1.8 vs 28.2±4.8 (p<0.0001) with ADP, and 17.0±5.2 vs 36.8±5.1 (p<0.02) with Collagen; the percent reductions of pAKT were 3.2±4.2 vs 43.1±3.5 (p<0.0001) with Collagen, and 5.4±5.2 vs 32.1±6.8 (p<0.004) with NaA; the percent reductions of pERK-2 were 3.5±2.2 vs 25±8.2 (p<0.004) with Collagen, and 10.5±8.6 vs 40.2±9.5 (p<0.03) with NaA; the percent reductions of ROS production were 15.1±5.2 vs 41.2±7.5 (p<0.006) with NaA. In the presence of GLP-1 (9-36): the percent increases of the antiaggregating effects of SNP were 6.1±1.9 vs 25.6±3.0 (p<0.0001) with ADP, and 18.5±3.8 vs 32.7±4.1 (p<0.02) with Collagen; the percent reductions of pAKT were 4.8±3.1 vs 45.3±4.6 (p<0.0001) with Collagen, and 6.1±3.3 vs 35.9±7.3 (p<0.0001) with NaA; the percent reductions of pERK-2 were 4.9±2.0 vs 28±9.1 (p<0.004) with Collagen, and 12.6±8.1 vs 44.0±9.8 (p<0.02) with NaA; the percent reductions of ROS production were 17.1±8.3 vs 45.9±7.9 (p<0.03) with NaA. In the presence of Liraglutide: the percent increases of the antiaggregating effects of SNP were 5.7±2.5 vs 30.1±7.8 (p<0.001) with ADP, and 15.5±3.5 vs 33.5±4.4 (p<0.003) with Collagen; the percent reductions of pAKT were 15.9±5.7 vs 39.1±9.0 (p<0.03) with Collagen, and 20.1±5.7 vs 36.2±5.8 (ns) with NaA; the percent reductions of pERK-2 were 4.0±2.5 vs 27.9±8.8 (p<0.003) with Collagen, and 24.3±7.7 vs 39.2±6.8 (ns) with NaA; the percent reductions of ROS production were 26.1±5.4 vs 56.8±6.3 (p<0.001) with NaA.

Conclusions. T2DM is characterized by reduced effects of GLP-1 (7-36), GLP-1 (9-36) and Liraglutide to increase the antiaggregating effects of NO, and to reduce platelet activation of the signalling pathways PI-3K and MAPK and oxidative stress stimulated by agonists. These findings suggest that an impairment of GLP-1 action could play a role in platelet hyperreactivity responsible, at least in part, of the increased prothrombotic risk in T2DM.

UNDERUSE OF LIPID-LOWERING THERAPY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background. In both primary and secondary cardiovascular prevention, LDL cholesterol is the main therapeutic target. Statins are the most largely used drugs to reach LDL-c goals. Non-alcoholic fatty liver disease (NAFLD) is now widely considered as a condition associated with a high cardiovascular risk.
Aims. The aim of the study was to investigate the use as lipid-lowering statin therapy in patients with NAFLD, compared to patients without.
Materials and Methods. 443 consecutive subjects, referred for suspected metabolic disease, were enrolled. Routine laboratory tests were performed and anamnestic data collected. All patients underwent ultrasonography abdominal examination to establish the presence of NAFLD. Cardiovascular risk classes and LDL-c targets were defined according to 2011 ESC/EAS guidelines.
Results. NAFLD was diagnosed in 359 patients. Mean age was 55,4±13 in patienst with NAFLD and 53.9±11.6 in those without (P>0.05). Diabetes prevalence was 29,6% and 15.6%, respectively (P<0.001). Prevalence of coronary heart disease was 27.6%, 1.8% of patients had a cerebrovascular previous event, 3,2% had peripheral artery disease and 5.9% had chronic kidney failure. Based on current guidelines, in the whole population, 72.5% of patients had an indication for statin-use, but only 47.7% of the above were on statin-therapy. Indication was present in 73,1% of NAFLD patients and in 67,5% of control subjects (p=ns). In the same two groups, appropriate use of statins was observed respectively in 43% and 62,5% of patients (p=0,01). Among patients with the indication for satin therapy, those who did not take treatment had higher mean ALT (36.1±26.3 vs 30.4±18.8 mU/l, p<0.05).
Conclusions. Our findings show an underuse of statin therapy in high risk patients. The percentage of underuse is higher in patients with NAFLD compared to those without. Patients on statin treatment had lower ALT values confirming safety and low liver toxicity of statin treatment in patients with NAFLD. Finally, although NAFLD is considered a high cardiovascular risk condition, our findings confirm the concern of physicians about the prescription of statins in these patients.

SOTTOCLASSI LIPOPROTEICHE E OSSIDAZIONE LIPIDICA IN PAZIENTI CON SINDROME DI KLINEFELTER E IN UOMINI E DONNE SANI DI PARI ETÀ

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Introduzione. Nei pazienti con sindrome di Klinefelter, anomalia numerica dei cromosomi sessuali con cariotipo più frequente 47XXY, vi è un significativo aumento della morbilità e mortalità cardiovascolare solo in parte attribuibile ad una più elevata frequenza di sindrome metabolica.
Soggetti e Metodi. Sono stati valutati 30 soggetti con sindrome di Klinefelter, di età compresa tra i 16 e 44 anni, non in terapia ormonale sostitutiva, 30 maschi e 40 femmine di controllo di pari età. In ciascun soggetto sono stati valutati i dati antropometrici, colesterolo totale (CT), C-HDL, C-LDL, trigliceridi (TG), distribuzione delle lipoproteine secondo gradiente di densità all'ultracentrifugazione (DGUC) e concentrazione delle LDL ossidate (ox-LDL).
Risultati I pazienti con sindrome di Klinefelter presentano valori di CT (media±DS 206±37 vs 181±34 mg/dl, p<0,02), C-HDL (56±13 vs 50±10 mg/dl, p<0,05) e TG (mediana 130 vs 71 mg/dl, p<0,01) significativamente più elevati rispetto ai controlli maschi e livelli di CT (206±37 vs 170±26 mg/dl, p<0,001), C-LDL (122±35 vs 98±22 mg/dl, p<0,001) e TG (130 vs 61 mg/dl, p<0,001) significativamente più elevati rispetto ai controlli femmine. Confrontando la distribuzione delle lipoproteine secondo DGUC, abbiamo riscontrato un aumento significativo (p<0,05) di colesterolo nelle frazioni HDL, VLDL ed LDL più leggere, associato ad una riduzione significativa (p<0,05) del colesterolo nelle LDL dense, rispetto ai controlli maschi; rispetto ai controlli femmine nei pazienti Klinefelter è emerso un aumento significativo (p<0,05) di colesterolo nelle frazioni VLDL ed LDL più leggere. Non ci sono differenze significative per quanto riguarda le ox-LDL tra pazienti con sindrome di Klinefelter e controlli sia maschi che femmine.
Conclusioni. lo studio suggerisce che nei pazienti con sindrome di Klinefelter un assetto lipidico caratterizzato da un aumento di TG plasmatici ed in particolare, rispetto ai controlli sani, della concentrazione del colesterolo nelle IDL e VLDL, lipoproteine proaterogene, possa contribuire all'aumento di morbilità e mortalità cardiovascolare osservato in questi pazienti.

REDOX STATUS HOMEOSTASIS AND CLOT ALTERATIONS IN WOMEN WITH PLACENTA-MEDIATED DISORDERS

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Pre-eclampsia is an early or late - onset multifactorial placenta - mediated disorder characterized by high blood pressure and proteinuria. Conformational placenta abnormalities or pre-existing patient pathology have been suggested as the main pathogenetic mechanisms. Our recent investigations showed marked alterations in fibrinogen functioning in pre-eclamptic women. In particular, a decreased fibrin susceptibility to plasmin-induced lysis has been described by monitoring, in polyacrylamide gel electrophoresis, the proteolysis of fibrin β chains at 0 h, 3 h, 6 h after plasmin addition. In the present study we investigated if ROS (Reactive Oxygen Species) could have a role in the observed fibrinogen functional alterations. Thus, we assessed blood redox status in 40 patients and 20 healthy and age-matched controls. Using fluorometric and colorimetric assays, oxidative stress markers and Total antioxidant capacity were assayed in plasma and the extent of carbonylation was determined in purified fibrinogen fractions. Intracellular ROS production was investigated in blood cell populations using a FACSCanto flow cytometer. Structural analysis of fibrin network was determined by Differential Interference Contrast microscopy. Our results clearly show a significantly higher erythrocyte- and leukocyte- derived ROS production and signs of oxidative stress in pre-eclamptic women. In patients total antioxidant capacity was strongly reduced in comparison to healthy controls. Patients' pu-

rified fibrinogen showed an increased extent of carbonylation in comparison with healthy controls. DIC revealed, in pre-eclamptic women, marked morphological and structural alterations in fibrin network. Another interesting finding is the significant and positive correlation between leukocyte derived-ROS and fibrin β chain intensity after 6 h of plasmin digestion.
In conclusion, our results suggest that enhanced ROS production and marked fibrinogen oxidative modification could have a role in determining fibrin resistance to plasmin-induced lysis. Further studies are currently in progress in order to understand the relationships between altered fibrinogen functioning and fibrinogen carbonylation level and to evaluate the effects of an antioxidant treatment in pre-eclamptic women.

ABCA1 AND HDL3 ARE REQUIRED TO MODULATE SMC TRANSDIFFERENTIATION IN A KLF4/MYOCARDIN-DEPENDENT PROCESS

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Up to 50% of foam cells in human lesions originates from smooth muscle cells (SMCs). Arterial SMCs express the ATP binding cassette (ABC) transporter ABCA1. Upon cholesterol loading, SMCs express macrophage markers and a phagocytic activity. To characterize the role of ABCA1 in this transdifferentiation process, we evaluated the phenotypic changes in SMCs isolated from wild type (WT) and ABCA1 knock out (KO) mice and how HDL3 affects these changes. Cholesterol loading causes the downregulation of the expression of typical SMC markers including ACTA2, alpha-tropomyosin and myosin heavy chain and increases the expression of macrophage-related genes such as CD68, Mac-2, SRB1, ABCG1 and ABCA1. HDL3 treatment in WT cells is able to normalize the expression of ACTA2, while the expression of macrophage-related genes is reduced. On the contrary, this preventive effect of HDL3 is completely lost in ABCA1 KO cells. Interestingly, the presence of HDL3 does not differently affect neutral lipid accumulation in WT or ABCA1 KO cells but stimulates phospholipids removal only in WT cells. Moreover, cholesterol loading reduces the expression of myocardin, a SMC specific-transcriptional coactivator involved in SMC differentiation, by up to 55% ($p<0.01$ vs respective control) in both cell types. HDL3 normalizes myocardin levels in WT cells while it does not have any effect in ABCA1 KO cells. The basal expression level of KLF4, a myocardin repressor, is almost double in ABCA1 KO cells compared to WT. After cholesterol loading, KLF4 is slightly reduced in WT cells, while its expression is halved in ABCA1 KO cells. HDL3 restores KLF4 to basal levels in KO cells, but it further reduces them in WT cells. These results indicate that HDL3, interacting with ABCA1, activate myocardin and modulate SMCs transdifferentiation in WT cells. The absence of ABCA1 in the KO SMCs blocks this regulatory pathway.

STATIN THERAPY REDUCES PHOSPATE LEVELS IN DIALYSIS PATIENTS: RESULTS FROM THE EPIDEMIOLOGICAL VITAMIN K ITALIAN STUDY (VIKI STUDY)

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Introduction and Aim. Elevated plasma phosphate levels are associated with increased cardiovascular morbidity and mortality in chronic kidney disease (CKD) patients. The aim of the study was to investigate a new potential use of statins in the treatment of hyperphosphatemia in hemodialysis CKD patients originally recruited in the VIKI Study.
Patients and Methods. 387 patients on hemodialysis since at least 1 year were included in the multicenter, cross-sectional, observational VIKI Study, conducted in 18 dialysis centers in Northern Italy. Serum concentrations of phosphate, 25(OH)vitamin D, vitamin K components (K1, MK4, MK5, MK6 and MK7), osteocalcin (bone Gla Protein, BGP) and matrix Gla protein, routine biochemistry including lipid profile were measured.
Results. 33% of patients (n=126) was on statin therapy. Patients on statin showed significantly lower HDL cholesterol (mean±SD: 40.4±11.1 vs 43.3±13.4 mg/dl, p=0.0473), and higher plasma triglyceride levels (median: 164.0 vs 142.0 mg/dl, p=0.0041) than those not on statin. Plasma phosphate levels were significantly lower in patients on statin therapy (4.57±1.12 vs 4.86±1.33 mg/dl, p=0.0365) compared to those not on statin. Concentrations of 25(OH)vitamin D were significantly reduced (median: 26.0 vs 30.7 nmol/l, p=0.0198), while plasma MK7 levels were significantly increased (median: 1.16 vs 0.84 ng/ml, p=0.0241) in subjects on statin therapy. In the multiple logistic regression analysis with plasma phosphate levels dichotomized according to the median value 4.6 mg/dl as outcome, the model adjusted for BMI, angina, LDL, BGP and antibiotics showed that statin therapy was significantly associated with lower plasma phosphate levels (OR 0.61, 95%CI 0.38-0.98, p=0.0411).
Conclusions. To our knowledge this is the first evidence of an association between lipid-lowering therapy with statins and reduced plasma phosphate levels in hemodialysis CKD patients. This observation remains to be further investigated in prospective studies in order to support a potential additional indication of statin therapy in the prevention of cardiovascular events both in CKD patients and in general population.

ASSOCIATION BETWEEN SELF-REPORTED SNORING AND METABOLIC SYNDROME: DATA FROM THE BRISIGHELLA HEART STUDY

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Our study aims to evaluate the association between self-reported snoring and metabolic syndrome among Brisighella's population. We analyzed 731 subjects, equally distributed between male and

female, from the 2012 cohort of the Brisighella Heart Study. Snoring, physical activity and smoking habit were recorded by using a questionnaire during the visit. We followed the National Cholesterol Education Program's Adult Treatment Panel III criteria for the definition of metabolic syndrome. The prevalence of snoring was 50.2% (n=367), the non-snorers accounted for 49.8% (n=364) and patients with the metabolic syndrome appeared to be 34.2% (n=250). Multivariate logistic regression analysis shows that, compared to non-snorers, those who report snoring are significantly associated with metabolic syndrome (OR 1,46387; 95% confidence interval (CI), 1,00035-2,14215) adjusting for BMI, sex, age, smoking status and physical activity. However, sex, smoking status and physical activity do not show significant correlation with the outcome assessed (all Ps >0.05) in the already built logistic model, therefore we built a new model that excludes these predictors, and the odds ratio does not change significantly (OR 1,47667; 95% CI, 1,01975-2,13833). During the collecting of medical history, patients who report sleep-disordered breathing such as snoring are more likely to have a cluster of metabolic disorders that are clinically defined as metabolic syndrome.

PREVALENCE AND CHARACTERISTICS OF THE USE OF LIPID-LOWERING AGENTS IN A POPULATION OF ELDERLY HOSPITALIZED PATIENTS: THE FINDINGS FROM THE REPOSI (REGISTRO POLITERAPIE SOCIETÀ ITALIANA DI MEDICINA INTERNA) STUDY

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Elderly patients often undergo polypharmacological treatment, with a high risk of adverse events and drug interactions. The indication to lipid lowering treatment in particular is quite controversial, and information on the actual use of such drugs is limited. AIM of the present study was to characterize the use of lipid lowering agents in a population of complex in-hospital elderly patients.
Methods. We analyzed the data from 102 Units of Internal Medicine or Geriatrics within the REPOSI (REgistro POliterapie della Società Italiana di Medicina Interna) study, in the 2010 and 2012 patients cohorts. 2171 subjects older than 65 were enrolled (1057 males, 1114 females, mean age 78.6 yr). Lipid lowering treatment was correlated with the clinical profiles, including comorbility markers and polytherapy.
Results. The patients treated with lipid lowering drugs were 508 (23.4%) with no gender difference. Regarding statin treatment, atorvastatin (39.3%) and simvastatin (34.0%) were the most used drugs. The prevalence of drug use was higher in patients under polypharmacological treatment (≥5 drugs) and with a higher comorbility (CIRS) score. In treated patients the percent cardiovascular risk according to Framingham's equation was higher. At logistical regression analysis coronary heart disease, peripheral vascular disease and hypertension were significantly correlated with hypolipidemic drug use, whereas age was inversely correlated. Diabetes was not associated with treatment.
Conclusions. In this in-hospital cohort the use of lipid lowering agents was mainly driven by the clinical history of the patient and associated with the presence of cardiovascular conditions (hypertension, coronary heart disease, peripheral vascular disease) but not with diabetes. Most frequently used drugs do not include pravastatin, the only evidence-based statin in primary prevention in the elderly, and fluvastatin, which claims a better pharmacometabolic profile. Increasing age seems to associate with a lesser prescription rate, reflecting a cautiousness behavior towards a potentially toxic treatment regimen.

LA SINDROME METABOLICA CONDIZIONA NEGATIVAMENTE IL RAGGIUNGIMENTO DEI TARGET TERAPEUTICI DEL COLESTEROLO LDL NEL PAZIENTE HIV+

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Introduzione. La terapia antiretrovirale ha contribuito a ridurre in modo rilevante la mortalità AIDS-correlata; tuttavia, la patologia cardiovascolare (CV) su base arteriosclerotica continua a rappresentare una importante causa di morbilità e mortalità nei pazienti HIV+. L'elevata prevalenza di fattori di rischio CV tradizionali nei pazienti HIV+ contribuisce all'incremento del loro rischio CV; quest'ultimo però sembra essere sottostimato dai comuni algoritmi di calcolo del rischio CV globale. Non includere la Sindrome Metabolica (SM), assai prevalente tra i pazienti HIV+, tra i fattori di rischio annoverati nella stima del rischio CV globale potrebbe in parte motivare la sottostima del rischio CV reale di questa categoria di pazienti. Obiettivo del nostro studio è stato quantificare il rischio CV globale, la prevalenza della SM e la distanza dai target raccomandati di LDL colesterolo in pazienti HIV+.
Metodi. Abbiamo quantificato i fattori di rischio CV tradizionali, la prevalenza della SM e la distanza dai target di LDL colesterolo raccomandati dal NCEP ATP-III in 350 pazienti HIV+. Nei pazienti HIV+ con SM, il rischio CV stimato è stato incrementato di una classe ed è stata quindi rivalutata la distanza dai target di LDL colesterolo modificati in funzione della riclassificazione del livello di rischio.
Risultati. Dalla stima dei fattori di rischio CV nella popolazione esaminata è emerso che l'85% dei pazienti HIV+ era portatore di almeno un fattore di rischio ed il 60% di almeno due. La SM era presente nel 23% dei pazienti HIV+ in terapia antiretrovirale. Solo il 9% dei pazienti era in terapia con statine, mentre il 24% non raggiungeva il target di LDL colesterolo raccomandato dal NCEP ATP-III. La percentuale di pazienti a basso rischio CV non a target per il colesterolo LDL era pari all'8%; questa percentuale raggiungeva il 70% tra i pazienti a rischio CV molto alto. La percentuale di pazienti non a target con un rischio CV riclassificato da basso-moderato a moderato-alto per la presenza della SM è passata dal 14% al 41%.
Conclusioni. Nei pazienti HIV+ la prevalenza di fattori di rischio CV isolati od in associazione (es. SM) è particolarmente elevata. La presenza della SM, aumentando il livello di rischio CV non quantificato dalle comuni carte di rischio, potrebbe essere impiegata per riclassificare il livello di rischio del paziente HIV+ e suggerire nuovi target di LDL colesterolo da raggiungere. La riclassificazione del rischio CV dettato dalla presenza della SM potrebbe richiedere un approccio più aggressivo nel controllo dei livelli di LDL colesterolo dei pazienti HIV+.

LA FUNZIONE ENDOTELIALE DEL PAZIENTE HIV+ È INFLUENZATA NEGATIVAMENTE DALLA COESISTENZA DI SINDROME METABOLICA E MICROALBUMINURIA

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Introduzione. L'infezione da HIV è spesso complicata da disfunzione renale. L'aumento del rapporto tra concentrazione urinaria di albumina e creatinina (ACR) è considerato un indicatore precoce di danno renale oltre che un predittore di aumentato rischio cardiovascolare (CV). La ridotta vasodilatazione flusso-mediata dell'arteria brachiale (bVFM) è un valido indicatore di disfunzione endoteliale e di rischio CV. Obiettivo dello studio è stato valutare l'esistenza di una correlazione tra ACR e bVFM in pazienti HIV+ con normale filtrazione glomerulare.
Metodi. ACR, bVFM e fattori di rischio CV tradizionali sono stati valutati in 160 pazienti con infezione da HIV e normale filtrazione glomerulare. La correlazione tra ACR e bVFM è stata valutata dopo correzione per molteplici fattori confondenti, quali diabete, ipertensione, sindrome metabolica (SM) e terapia antiretrovirale.
Risultati. Dalla misura dell'ACR è emersa una elevata prevalenza di danno renale precoce tra i pazienti HIV+: il 25% di questi presentava infatti valori di ACR superiori a 20 mg/g. I pazienti HIV+ con diabete od ipertensione arteriosa avevano livelli di ACR più alti ($p<0,01$) rispetto a quelli senza diabete o ipertensione. Il 22% dei pazienti portatori di un singolo componente della SM presentava un'ACR alterata, mentre tra i pazienti con cinque componenti della SM l'ACR è risultata alterata nel 50% di casi. Nella popolazione di studio, è stata documentata una correlazione negativa tra ACR e bVFM, indipendente dalla presenza di fattori confondenti (beta=-0.17, p=0.04); nei pazienti senza diabete ed ipertensione, la correlazione tra ACR e bVFM è stata confermata (rho=-0.28, p=0.005). Il maggior grado di correlazione negativa tra ACR e bVFM è stato riscontrato tra i pazienti con SM (rho=-0.52, $p<0,001$). I pazienti con SM ed elevati valori di ACR avevano valori di bVFM più bassi rispetto a quelli con nessuna od una delle due condizioni ($p<0,05$ per entrambi i confronti).
Conclusioni. Elevati valori di ACR si associano a disfunzione endoteliale indipendentemente dalla coesistenza di fattori confondenti quali il diabete, l'ipertensione, la SM e la terapia antiretrovirale. ACR elevata e presenza della SM esercitano un effetto negativo sinergico sulla bVFM. Lo screening dei singoli componenti della SM e la quantificazione dell'ACR dovrebbero fare parte dei protocolli di gestione dei pazienti con infezione da HIV.

TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT NEPHROPATHY: LIPOPROTEIN SUBCLASS DISTRIBUTION, OXIDIZED LDL AND PERIPHERAL ARTERY DISEASE

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Background and Aims. Type 2 Diabetes (DM2) is associated to an increased cardiovascular mortality and morbidity (CVD). The presence of diabetic nephropathy (DN) further increase CVD and the cause(s) are still to be fully elucidated. We investigated quantitative as well as qualitative lipoprotein profiles, oxidized LDL (ox-LDL) concentration and the presence of peripheral artery disease (PAD) in DM2 patients with and without DN.
Subjects and Methods. 47 patients with DM2, 22 patients with DN (eGFR<60 ml/min or albuminuria) and 25 without ND were studied. Clinical and anthropometric data, severity of DN and glomerular filtration rate (by the Cockcroft and Gault formula), full lipid profile, plasma ox-LDL, LDL density and lipoprotein subclass distribution by ultracentrifugation, presence of subclinical atherosclerosis (Ankle-Brachial-Index) were evaluated.
Results. Type 2 diabetic patients with DN were characterized by a significantly higher plasma triglycerides (168±91.5 vs 112±63.4 mg/dl, p=0.029), cholesterol in the TG-rich lipoproteins (VLDL, IDL), ox-LDL (65.6±10.7 vs 53.9±0.4 mg/dl) and denser LDL (Rf 0.324±0.08 vs 0.351±0.11, p=0.048) than non DN DM2 patients.
A higher percentage of DM2 with ND had PAD (ABI<0.9: 68% vs 18% DN vs no DN, $p<0.01$) with worse ABI score (0.86±0.06 vs 1.09±0.04 p=0.037), and a greater number of patients with claudicatio intermittens (45% vs 16%, p=0.044).
By linear regression, ox- LDL levels were significantly associated with non-HDL cholesterol, VLDL cholesterol, and total, VLDL and IDL triglycerides, glycated hemoglobin and LDL density.
Conclusion. Presence of nephropathy in type 2 diabetic patients is associated with more severe PAD and a significant worsening of the pro-atherogenic traits (dense LDL, TG-rich lipoprotein cholesterol, ox-LDL) of the lipid phenotype seen in type 2 diabetic patients without nephropathy.

FAMILIAL HYPERCHOLESTEROLEMIA IS ASSOCIATED WITH IMPAIRED CD4+ T CELL DIFFERENTIATION AND FUNCTION: FOCUS ON THE IMMUNOMETABOLIC EFFECT OF LDL-R

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Adaptive immune response has recently gained attention during atherosclerosis due to the correlation found between T effector memory cell (TEM) expansion, reduced T naïve cells (TN) and the progression of the disease. Whether this correlation is merely the consequence of increased levels of plasma cholesterol is debated. Here we aimed at investigating whether key players linking systemic and cellular lipid metabolism, such as the LDL-R, affect the differentiation and functionality of CD4+ T cells in animal models and in humans.
First we characterized CD4+ T cell differentiation in LDLR KO mice compared to WT by flow cytometry analysis. While under resting condition, differentiation of CD4+ T cells in secondary lymphoid organs was similar in LDLR KO and WT, anti-CD3 and anti-CD28 stimulated CD4+ T cells of LDLR KO, isolated from lymph nodes, proliferated more compared to WT. Following 16 weeks of western type diet, LDLR KO mice presented significantly reduced circulating levels of TN (CD4+CD44-CD62L+) and increased TEM (CD4+CD44+CD62L-) compared to WT, which were further correlated with

the extension of atherosclerotic plaques in their aortic sinus. In a different in vivo setting, such as the rejection during skin allograft transplantation, where a proper effector T cell response is critical, the draining lymph nodes of the graft in the LDLR KO also presented increased levels of TEM compared to WT. Of note in parallel with increased TEM levels also an increased expansion of T regulatory (Treg) was observed, in line with our previous findings in animal models and in humans with cardiovascular diseases. To investigate whether these observations could be the consequences of an impaired Treg functionality we focus our attention on patients affected by familiar hypercholesterolemia (FH) (all heterozygote for LDL-R). We observed that FH patients presented significantly increased levels of circulating TEM compared to age and sex matched controls, which was paralleled by increased proliferation of peripheral blood mononuclear cells (PBMC) in vitro after antigenic stimulation. Similarly to what reported above, circulating levels of Treg were significantly increased in FH patients compared to age and sex matched controls, further suggesting that also in humans the expansion of TEM might be the consequence of an impaired functionality of Treg. Together our data propose a novel connection between cholesterol metabolism and immune response, showing that LDLR deficiency in CD4+ T cells leads to an expansion of TEM as a consequence of dysfunctional Treg cells thus supporting the increased immune response observed during atherosclerosis.

CARDIOVASCULAR DISEASE (CVD) RISK FACTORS IN A POPULATION OF CHILDREN WITH SEVERE HYPERCHOLESTEROLEMIA

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Aim. to determine the cardiovascular risk profile in children with severe primitive hypercholesterolemia at the first access to our Lipid Clinic and to estimate the prevalence of genetically-confirmed familial heterozigous hypercholesterolemia.
Methods. 186 severely hypercholesterolemic children (median age 8,7 y, 80 male/106 female) with positive history for hypercholesterolemia and/or premature CVD and no secondary causes of hypercholesterolemia, were evaluated for: twelve-hour-fasting blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and Triglycerides (Try) by enzymatic method; apolipoprotein B (apoB) and apolipoprotein A1 (apoA1) by immunoturbidimetric assay; Lipoprotein(a) (Lpa) levels by nephelometry and genetic analysis for LDL-receptor gene (heFH) by polymerase-chain-reaction. None was on pharmacological treatment.
Statistics. Student's t test or Mann-Whitney test for independent samples.
Results. The 186 patients were divided in two groups: 57 (31%) had a mutation on LDL receptor gene (heFH+) and 129 (69%) had no mutation (heFH-). The lipid profile (mg/dl, mean±ds) in the heFH+group and heFH- was, respectively: TC 267.98±54.93 vs 217.80±47.34 ($p<0.001$), LDL-C 195.49±54.62 vs 142.81±47.42 ($p<0.001$), HDL-C 55.02±11.49 vs 57.07±14.45 ($p=0.345$), Try 70.21±30.89 vs 83.56±50.05 ($p=0.119$), apoB 124.18±29.51 vs 98.46±31.87 ($p<0.001$), apoA1 133.56±21.21 vs 138.78±24.65 ($p=0.167$), apoB/apoA1 0.93±0.32 vs 0.74±0.33 ($p=0.015$), Lp(a) 19.75±26.57 vs 27.62±32.22 ($p=0.102$).
Conclusions. In our population we found that children with familial heterozygous hypercholesterolemia have a worse CVD risk profile (more elevated TC, LDL-C, apoB levels; apoB/apoA1 >0.9). The genetic analysis of the LDL-receptor gene is important in childhood to individuate children who have to undergo adequate follow up and early pharmacological treatment.

METABOLIC AND LIPOPROTEIN ABNORMALITIES IN PATIENTS WITH KLINEFELTER SYNDROME

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Background and Aims. Patients with Klinefelter Syndrome (KS) have a reduced life expectancy associated with an increased morbidity and mortality due to cardiovascular disease (CVD). There is still controversial evidence on which risk factor(s) might contribute significantly to the increased CVD risk, and potentially represent new therapeutic targets to modulate CVD in KS. The aim of this study was to investigate the potential contribution of quantitative and qualitative lipoprotein abnormalities, including LDL density and oxidation, to the CVD risk of patients with KS naïve of any prior hormone replacement therapy. We focused on lipoprotein phenotypes in KS patients according to their waist circumference.
Subjects and Methods. Anthropometric data and fasting blood samples, lipid profile, qualitative lipoprotein analysis by density gradient ultracentrifugation (DGUC), plasma oxidized LDL (ox-LDL), fasting glycaemia, glycated hemoglobin, HOMA index, LH, FSH, testosterone, SHBG and TSH were analyzed in 30 KS patients.
Results. Klinefelter patients have a waist circumference (WCirc) suggestive (IDF criteria) of central obesity (98.9±18 cm). According to WCirc tertiles, patients in the two upper tertiles (WCirc >91.3 cm) had a proatherogenic lipid profile characterized by significantly higher TG, lower HDL-C, increased prevalence of dense LDL (0.369 vs 0.392 Rf; $p<0.05$) and ox-LDL (61.1±16 vs 46.8±10 U/L, $p<0.05$) as compared with KS in the lower tertile (WCirc <91.3 cm). Analysis by DGUC of the lipid profiles in the upper vs lower tertiles of WCirc, fully confirmed this observation. We found an inverse correlation between ox-LDL and LDL density ($r=-0.409$, $p=0.025$). Ox-LDL levels were significantly associated with the dense LDL fractions by DGUC (fractions 9-12; $p<0.05$). By multiple logistic regression analysis, low levels of plasma testosterone were significantly associated with an increased WCirc [OR 0.73 (0.54-0.97), $p=0.029$].
Conclusions. In KS low testosterone levels are associated with an increased WCirc that is characterized by a proatherogenic lipid profile.

EFFETTO DELLA CHIRURGIA BARIATRICA SU BIOMARKERS VASCOLARI IN PAZIENTI OBESI NORMOTESI NON-DIABETICI

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Metodi. Sono stati reclutati 25 pazienti con obesità severa (19 donne, età 44±11 anni, BMI 45±7 kg/m^2) normotesi, non-diabetici e con normale funzione renale. Una valutazione vascolare multidi-

strettuale è stata eseguita prima e 12 mesi dopo l'intervento di chirurgia bariatrica. Sono state valutate: la rigidità aortica mediante pulse wave velocity carotido-femorale (PWV), la funzione endoteliale come flow-mediated dilation (FMD), lo spessore medio-intimale carotideo (cIMT) e la distensibilità carotidea (DC) mediante ultrasuoni, e l'emodinamica renale mediante indici di resistenza renali basali (RI) e dinamici (DRIN, dopo somministrazione di nitroglicerina 25 mcg s.l.). Il flusso plasmatico renale pre-intervento è stato valutato mediante infusione di 123I-orto-iodo-ippurato.
Risultati. Al momento 13 pazienti hanno completato il follow up a 12 mesi, con una riduzione del BMI da 45±7 a 32±6 kg/mq (p<0.001), della pressione arteriosa (PA) media da 93±11 a 86±6 mmHg (p=0.05) e della frequenza cardiaca da 69±9 a 59±9 bpm (p=0.01). HDL risultava aumentato (da 44±10 a 52±9, p=0.04) e i trigliceridi ridotti (da 121±57 a 76±27 mg/dl, p=0.01), mentre la glicemia a digiuno (da 95±20 a 87±8 mg/dl, p=0.22), il colesterolo totale (da 179±26 a 172±40 mg/dl, p=0.61) e la creatinina (da 0.71±0.14 a 0.69±0.11, p=0.84) erano invariati. Si è verificato un significativo aumento della FMD (da 4.8±2.1 a 6.7±2.3%, p=0.002) e una significativa riduzione di PWV (da 8.3±1.1 a 7.5±1.2 m/s, p=0.04), RI (da 0.63±0.06 a 0.58±0.06, p=0.05) mentre IMT (da 0.71±0.16 a 0.64±0.14, p=0.23), DRIN (da -5.3±4.3 a -6.2±4.1%, p=0.80) e DC (da 27.2±6.7 a 32.4±10.1, p=0.15) sono rimasti invariati. Le variazioni di PWV, ma non quelle di FMD e RI, erano correlate alle variazioni di PA media (r=0.76, p=0.006). I Δ PWV, FMD e RI non erano correlati ai Δ BMI, HDL o trigliceridi. Il flusso plasmatico renale pre-intervento era correlato positivamente a RI pre-intervento (r=0.65, p=0.03) e negativamente al Δ RI (p=-0.63, r=0.04).
Conclusioni. La chirurgia bariatrica è in grado di migliorare la funzione endoteliale e l'emodinamica renale anche in individui con obesità severa normotesi e non diabetici. Questo miglioramento è indipendente dall'effetto sulla pressione arteriosa e sul profilo lipidico. L'incremento di RI nei grandi obesi sembra essere funzionale e reversibile.

EFFICACIA E TOLLERABILITÀ DI LOMITAPIDE: COME GESTIRE GLI AUMENTI DI DOSAGGIO IN PAZIENTI CON EFFETTI COLLATERALI. CASE REPORT

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L'ipercolesterolemia familiare (FH) omozigote (HoFH) o doppio eterozigote (compound, 2HeFH) è notoriamente resistente al trattamento e difficilmente riesce a raggiungere i target terapeutici (LDL-C<100) nonostante terapia con statine ai massimi dosaggi tollerati e anche in associazione a ezetimibe (Eze). L'LDL-Aferesi (LDL-A) offre un'ulteriore possibilità, ma è procedura invasiva e a volte mal tollerata. Per questi pazienti è disponibile da circa un anno la Lomitapide (Lo) con le indicazioni riportate in G.U. (diagnosi clinica di HoFH o 2HeFH) ma la casistica nazionale è ancora molto limitata e l'esperienza sull'efficacia e tollerabilità del farmaco deve ancora basarsi anche sull'aneddotica. Riportiamo il caso di una paziente difficile da stabilizzare al trattamento a causa di fastidiosi effetti collaterali (sindrome diarroica). Donna di 66 anni, non fumatrice, BMI =23,4; affetta da FH (nel 2009 LDL-C =423 mg/dL in trattamento con Eze/simva 10/40 mg/die) caratterizzata da familiarità positiva, arco corneale e xantomatosi tendinea (tendine d'Achille e dorso delle mani) (Dutch Lipid Score =21; mutazione c.304C>T del LDLR) e complicata da placche carotidee bilaterali (TEA nel 2013). Ipertensione di recente insorgenza. Lieve epatosteatosi (stabile). È seguita presso il nostro Centro dal 2012; a causa di valori persistentemente elevati (LDL-C=183) simva 40 fu sostituita con atorva 40 mg in associazione estemporanea con Eze e fu prescritta LDL-A ogni 2 settimane, sempre mal tollerata per effetti collaterali e problemi logistici. Nel novembre 2014 ha iniziato Lo 5 mg on top al suo schema terapeutico. In associazione: vit. E (400 U), n-PUFA (1 g/die) e dieta 1.200 Kcal (18% lipidi). Dopo un mese (LDL-C pre-LDL-Aferesi =263 → 169; -38%; nuovo farmaco ben tollerato) è passata a Lo 10 mg/die ma ha subito accusato diarrea, tale da indurla alla sospensione del trattamento e anche dell'LDL-Aferesi. Ha poi ripreso Lo alternando 5 e 10 mg con ottima efficacia (LDL-C =115; -56%) ma persistenza di diarrea, migliorata dopo sospensione di Eze; permangono saltuari episodi, controllati con loperamide 2 mg. L'attuale schema di trattamento è Lo 10 mg e Atorva 40 mg/die. Ultimo LDL-C =140 mg/dL (-47%). Le transaminasi epatiche non hanno mai superato i valori di norma. La paziente ha sempre avuto un'ottima aderenza alla terapia e da circa 2 mesi sono scomparsi gli xantomi tendinei alle mani. La compliance del paziente e l'attitudine del medico a voler comunque ricercare lo schema di trattamento meglio tollerato possono migliorare di molto il profilo lipidico e la qualità di vita di questi pazienti ad alto rischio e di difficile gestione clinica.

OBIETTIVI TERAPEUTICI, CONTROLLO DELLA SPESA ED EVOLUZIONE DELLE CONOSCENZE SCIENTIFICHE: COME È CAMBIATA LA PRESCRIZIONE DEI FARMACI IPOLIPIDEMIZZANTI NELLA COMUNE PRATICA CLINICA DI UN CAMPIONE DI MMG ABRUZZESI NEGLI ULTIMI 10 ANNI

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Background. Pur restando all'interno delle indicazioni d'uso di ciascun farmaco, l'appropriatezza prescrittiva dovrebbe essere finalizzata al raggiungimento di obiettivi terapeutici e, eventualmente, modificarsi nel tempo in funzione delle nuove conoscenze.
Scopo. Valutare se e come si siano modificate le abitudini prescrittive della terapia ipolipidemizzante negli ultimi 5 anni, in funzione delle varie modifiche della nota 13 e delle nuove evidenze scientifiche.
Metodi. Abbiamo analizzato, in maniera retrospettiva, i dati completi di 301 pazienti, estratti automaticamente mediante software Quick Openetica dai database di un gruppo di 40 MMG della ASL di Chieti nel periodo compreso tra 2005 e 2015. Tali dati, una volta criptati, sono stati inviati al nostro server, trasferiti su foglio di lavoro excel ed elaborati per l'analisi statistica. I dati dei pazienti erano continuativi e valutabili nell'arco dei 10 anni considerati. Abbiamo considerato il 2005 (anno di commercializzazione dell'associazione ezetimibe/simvastatina) come punto di partenza per il riferimento dell'efficacia della terapia ipolipidemizzante in termini di raggiungimento del target LDL-C. Abbiamo poi valutato i dati clinici dei pazienti tra il 2010 e il 2015 e l'andamento delle prescrizioni farmacologiche in considera-

zione delle diverse condizioni di mercato e delle norme regolatorie. Dopo il 2010, le prescrizioni farmacologiche su questi pazienti sono state monitorate ogni 6 mesi fino al giugno 2015.
Risultati. Nel 2010 le caratteristiche demografiche e cliniche dei pazienti erano: età media =72 aa; M=70, F=72; BMI medio =36; M=38, F=33; CHD=43,5%; DM=46%; IFG=30%; S. Met.=24%). RCG molto alto (43,6%), alto (43,6%), o moderato (12,6%). I pazienti trattati e a target LDL-C erano il 38,1% (rispetto al 26,3% del 2005) mentre il 61,9% non era efficacemente trattato o non lo era affatto. Nel 2015 i pazienti a target erano il 54,5%, dato che si discosta da quello medio regionale (39,6%). Nel periodo di riferimento si è assistito a un lieve calo delle prescrizioni di atorvastatina (38% vs 35%) e a un lieve aumento di rosuvastatina (14% vs 17%), dell'associazione (5% vs 8%) e dell'ezetimibe in monoterapia o in associazione estemporanea (non ammessa a rimborso nel 2010, 0% vs 3% nel 2015). Per qualunque molecola, i dosaggi più prescritti erano quelli medio-bassi (es. 10-20 mg per atorva; 5-10 per rosuva) in molti casi con una evidente inerzia prescrittiva.
Conclusioni. Si osserva una sostanziale stabilità nelle prescrizioni di atorva (priva ormai di brevetto) controbilanciata da un aumento di prescrizioni di rosuva ed ezetimibe (in associazione fissa o estemporanea), ma con una riduzione netta della spesa totale.
L'andamento del nostro campione dimostra che il monitoraggio delle prestazioni (attraverso strumenti di controllo dati anche in remoto e di self-audit) induce nei MMG una maggiore appropriatezza prescrittiva, con un'attenzione sia al risparmio sia alle nuove evidenze scientifiche. Ulteriori sforzi, però, devono ancora essere fatti, perché circa la metà dei pazienti (e tra essi la maggior parte di quelli a più alto rischio) non sono adeguatamente trattati.

LIVER-SPECIFIC DELETION OF THE PPAP2B GENE WORSENS ATHEROSCLEROSIS IN APOE-/- MICE

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Lipid phosphate phosphatases (LPPs) are integral membrane proteins that catalyze the dephosphorylation of a broad panel of lipid substrates. The LPP family is composed by three enzymes in mammals: LPP1, LPP2 and LPP3, coded by three independent genes named PPAP2A, PPAP2C and PPAP2B, respectively. The expression of LPP2 mRNA is found mainly in brain, pancreas and placenta, whereas LPP1 and LPP3 mRNAs appear quite ubiquitous.
A great interest around PPAP2B/LPP3 was recently raised by the results of genome-wide association studies (GWAS) that identified PPAP2B as a gene playing a role in coronary artery disease (CAD) susceptibility. Aim of the study was to investigate the effect of Ppap2b deletion on atherosclerosis development in an athero-prone mouse model. Since constitutive deletion of Ppap2b in mice leads to embryo-lethality, conditional Ppap2b hepatocyte-specific null mice were generated by crossing floxed Ppap2b mice (Ppap2bf/f) with animals expressing the Cre recombinase under the control of the Albumin promoter. These mice were then crossed with apoE-/- mice, obtaining Ppap2bf/fApoE-/-Alb-Cre+ mice and Ppap2bf/fApoE-/-Alb-Cre- mice as controls. The liver was chosen as target organ of Ppap2b deletion, being the main source of circulating plasma lipids and lipoproteins, which importantly contribute to the atherosclerosis process. Mice of both genotypes were fed chow or Western diet for 40 and 12 weeks, respectively. At the end of the dietary treatments, atherosclerosis development was evaluated and plasma lipidomic analysis was performed. Compared with Cre recombinase negative mice, Ppap2b mRNA expression dropped over five-folds in the liver of Ppap2bf/fApoE-/-Alb-Cre+ mice. The presence of a residual Ppap2b functional gene in liver could be explained by the presence of non-hepatocyte cell types, such as sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells and lymphocytes. Comparable expression levels were observed in all the other organs/tissues assayed in Ppap2bf/fApoE-/-Alb-Cre+ and Ppap2bf/fApoE-/-Alb-Cre- animals, demonstrating the high specificity of Albumin promoter expression and Cre recombinase activity. Hepatocyte-specific Ppap2b deletion was associated to an increased atherosclerosis progression: this result was observed both at the aortic sinus (4.8±1.5×105 μm^2 in Cre+ mice vs. 3.6±1.4×105 μm^2 in Cre- mice), and at the aorta (Arch: 45.84±5.92% in Cre+ mice vs. 24.59±6.3% in Cre- mice. Thoracic: 3.59±1.79% in Cre+ mice vs. 0.56±0.57% in Cre- mice. Abdominal: 4.37±2.25% in Cre+ vs. 4±4.37% in Cre- mice), when mice were fed Western diet, whereas no difference was found for atherosclerosis development between Ppap2bf/fApoE-/-Alb-Cre+ and Ppap2bf/fApoE-/-Alb-Cre- mice, when fed chow diet. With the aim of better understanding the impact of hepatic Ppap2b deletion on circulating lipids, possibly affecting atherosclerosis development, a plasma lipidomic analysis was conducted. When comparing Ppap2bf/fApoE-/-Alb-Cre+ and Ppap2bf/fApoE-/-Alb-Cre- mice fed chow diet, a significant increase of lactosylceramide in Ppap2bf/fApoE-/-Alb-Cre+ mice was observed. Western type diet, in both mouse lines, led to higher plasma concentrations of cholesteryl esters, sphingolipids and most glycerophospholipids compared with the chow diet. Interestingly, in Ppap2bf/fApoE-/-Alb-Cre+ mice only, an elevation of lysophosphatidic acid and triglycerides was observed. Lipidomic comparison between Ppap2bf/fApoE-/-Alb-Cre+ and Ppap2bf/fApoE-/-Alb-Cre- mice after Western diet feeding showed significant differences between the two mouse lines. Specifically, in Ppap2bf/fApoE-/-Alb-Cre+ mice, plasma levels of lipids playing a role in atherosclerosis development such as lactosylceramide, lysophosphatidic acid, globotriosylceramide and lysophosphatidylinositol were significantly increased, compared with Western-fed Ppap2bf/fApoE-/-Alb-Cre- mice. In conclusion, the present work demonstrates for the first time, in an animal model, the role of Ppap2b/LPP3 in atherosclerosis development and provides an experimental evidence for the clinical observation relating PPAP2B polymorphisms to CAD susceptibility. Additionally, the study indicates that hepatic Ppap2b deletion leads to alterations in the plasma levels of several minor lipid species, whose role in atherosclerosis has been proven, providing a molecular basis for the observed results.

DIFFERENCES OF LIPID PROFILE IN FH PATIENTS BEARING DIFFERENT MUTATION TYPES

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Introduction. Familial Hypercholesterolemia (FH) is characterized by increased LDL-cholesterol (LDL-c) levels and increased

cardiovascular risk. Autosomal dominant FH is caused by mutations in LDLR, APOB and PCSK9 genes; a recessive form of FH is caused by very rare mutations in LDLRAP1 gene. We aim to compare the lipid profile among the patients carrying different mutation types or mutations in the different genes
Materials and Methods. We enrolled 369 unrelated patients with a clinical diagnosis of FH. LDLR, PCSK9, LDLRAP1 genes and the exons 26 and 29 of APOB, were amplified and directly sequenced. Large rearrangements of LDLR were identified by SALSA MLPA kit (MRC-Holland).
Results. The screening revealed mutations in 249 patients showing a mutation rate of 67.5%. We found 70 different mutations in LDLR, 5 in APOB, 5 in PCSK9 and 1 in LDLRAP1 gene. Since splicing, nonsense, frameshift mutations or large deletions lead to a greatly modified protein, they are considered radical mutations. Levels of total-cholesterol (TC) and LDL-c gradually increased from patients without mutation (median TC=284 mg/dL; median LDL-c=194 mg/dL), carriers of missense mutations (median TC=307 mg/dL; median LDL-c=242 mg/dL), carriers of radical mutations (median TC=337 mg/dL; median LDL-c=261 mg/dL), homozygotes/compound heterozygotes (median TC=395 mg/dL; median LDL-c=292 mg/dL) with p<0.0001. Patients with missense mutations in the LDLR gene had higher TC levels (median TC=310 mg/dL) than patients with mutations in APOB or in PCSK9 (median TC=248 mg/dL) with p=0.025.
Conclusions. Since the mutations in the different genes, as well as the different mutation types are associated with different lipid profiles, the evaluation of the mutation type and of mutated gene could be useful for the patient management.

VITAMIN D STATUS IN RHEUMATOID ARTHRITIS: INFLAMMATION, ARTERIAL STIFFNESS AND CIRCULATING PROGENITOR CELL NUMBER

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Background. Suboptimal vitamin D status was recently acknowledged as an independent predictor of cardiovascular diseases and all-cause mortality in several clinical settings, and its serum levels are commonly reduced in Rheumatoid Arthritis (RA). Patients affected by RA present accelerated atherosclerosis and increased cardiovascular morbidity and mortality with respect to the general population. In RA, it has been reported an impairment of the number and the activity of circulating proangiogenic haematopoietic cells (PHCs), including CD34+, that may play a role in endothelial homeostasis. The purpose of the study is to investigate the association between vitamin D levels and PHCs, inflammatory markers, and arterial stiffening in patients with RA.
Methods. CD34+ cells were isolated from 27 RA patients and 41 controls. Vitamin D levels, C-reactive protein (CRP), fibrinogen, pulse wave velocity (PWV), and carotid intima-media thickness (cIMT) were also evaluated.
Results. CD34+ count (1.8±0.6 vs 2.5±0.9) and vitamin D levels (23±7.6 vs 31.7±5.2) were lower in RA patients as compared to controls, while fibrinogen, PWV, cIMT (all p<0.001) and CRP (p<0.05) were higher in RA patients. CD34+ cell number appeared to be associated with vitamin D levels (rs 0.706; p<0.001), and negatively correlated to fibrinogen (rs -0.546; p<0.01) and early atherosclerosis markers (PWV, p<0.01; cIMT, p<0.05); vitamin D levels appear also to be inversely associated to fibrinogen (rs -0.491; p<0.01).
Conclusion. RA patients with moderate disease activity presented with low vitamin D levels, low CD34+ cell count, increased PWV and cIMT; we found that vitamin D deficiency is associated to CD34+ cell reduction in peripheral blood, and with fibrinogen levels. This suggests that vitamin D might contribute to endothelial homeostasis in patients with RA.

EFFECTS OF ARMOLIPID PLUS ON SMALL DENSE LDL PARTICLES IN SAMPLE OF PATIENTS AFFECTED BY FAMILIAL COMBINED HYPERLIPIDEMIA

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Objectives. Familial Combined Hyperlipidemia (FCHL) is a common genetic disorder associated with high cardiovascular risk (CV), with elevated cholesterol or triglycerides levels or both, decrease in high density lipoprotein concentration, elevated apolipoprotein (apo) B and increased prevalence of atherogenic, small, dense low-density lipoprotein (sd-LDL) subfractions. Aim of this study was: to test sd-LDL changes with Armolipid Plus treatment in patients with FCHL diagnosis.
Design and Methods. After 4 weeks of standard diet, thirty patients with FCHL were included in an 8 week randomized, double-blind study and were taking, in addition to the standard diet, either Placebo or a nutraceutical combination that contains red yeast rice extract, berberine, policosanol, astaxanthin, coenzyme Q10, and folic acid (Armolipid Plus).
Results. Results in Placebo group showed no statistically significant differences in studied parameters; on the other hand, in Armolipid Plus group, statistically significant reductions differences were detected in BMI (p=0.010), LDL score (p=0.035) and an increase in mean LDL particle diameter (p=0.040) between week 0 and week 8. In addition, a comparison of studied parameters was performed between placebo and Armolipid Plus group: BMI (p=0.004), waist circumference (p=0.026), and diastolic blood pressure (p=0.024), LDL score (p=0.033) and mean LDL particle diameter (p=0.021) resulted statistically different between the two groups.
Conclusions. The main finding of this study is that association between a standard diet with Armolipid Plus is able to reduce small dense LDL score and increase LDL particle diameter in a group of Familial Combined Patients after 8 week of treatment.

DETECTION OF FAMILIES AT HIGH RISK FOR CARDIOVASCULAR DISEASE: SCREENING STRATEGIES CAN AND MUST START IN CHILDHOOD

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Background. Lipid screening in childhood is an issue of utmost importance for cardiovascular disease prevention, since hypercholesterolemia is one of the main modifiable risk factors for athero-

sclerosis and cardiovascular disease. Aim of this study is to find an effective screening strategy to detect families at high risk for cardiovascular disease.
Matherials and Methods. A questionnaire investigating the knowledge of lipid and cardiovascular disease issues was distributed to the parents of the newborns at Neonatology of Piacenza Hospital.
Results. 181 questionnaires have been collected so far (ongoing study). 39 couples of parents (21%) know their own lipid profile. 33 couples of parents (18%) know the correct normal values of total cholesterol. 61 couples of parents (33%) have first or second degree relatives with lipid disorders. 53 couples of parents (29%) have first or second degree relatives with premature cardiovascular disease. Considering the couples with positive family history for premature cardiovascular disease, only 11 out of 53 (20%) are aware of their own lipid profile. Considering the couples with positive family history for both premature cardiovascular disease and lipid disorders, 17 out of 25 (68%) have never had blood lipid screening done.
Conclusions. Collecting a problem-tailored and accurate family history seems to be a good strategy to detect high risk families, but the parents' poor awareness of the problem puts some limits to it. These preliminary data suggest that the percentage of adults with a positive family history for cardiovascular disease and/or lipid disorders and who are unaware of their own lipid profile is higher than expected. This implies that the number of undetected pediatric patients at high cardiovascular risk might be higher than expected. In this context, the identification of an effective and reliable screening strategy for cardiovascular disease in childhood is highly advisable.

CIGARETTE SMOKE CONDENSATE AND AQUEOUS EXTRACT DIFFERENTLY AFFECT THE INTERACTION BETWEEN ENDOTHELIUM AND MONOCYTES

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Cigarette smoke (CS) is a risk factor for cardiovascular disease and causes vascular endothelial cell (EC) dysfunction, a key event in atherogenesis. We recently demonstrated that Cigarette Smoke Condensate (CSC) induces human monocytes (HM) to release chemotactic factors that amplify the recruitment and transmigration of inflammatory cells through EC and stimulate the EC expression of adhesion molecules and MMPs, contributing to plaque progression. Moreover, incubation of EC with medium conditioned by HM treated with CSC (CMCSC), causes a change in cellular morphology and the shrinking of the cytoplasm (-50% vs control), as showed by visualizing the actin cytoskeleton with FITC-conjugated phalloidin. In the present study, we investigated the effects of the Aqueous Extract (AE) of CS, which contains water soluble components of the condensate and the gas and vapor phases, on HM and EC behavior. Cells were incubated with 10% AE (a not toxic concentration, as measured by the MTT assay), or with medium conditioned by HM alone (CHM) or exposed to AE (CMAE). In EC, incubation with AE decreased the expression of the adhesion molecules VCAM-1 and ICAM-1 (-50% and -15% vs control, respectively, $p<0.05$) and did not affect MMPs expression. Similarly to AE, CMAE caused a reduction of VCAM-1 by 65% vs CHM ($p<0.05$), whereas the MMP-2 expression was increased (+130%, $p<0.01$). AE reduced the expression of IL-8 by HM (-80%, $p<0.01$), while IL-1β, MCP1 and TNF-α were not affected.
At a morphological level, AE caused the shrinking of the EC cytoplasm (-55% vs control, $p<0.001$). The same occurred after treating cells with CHM, whereas CMAE induced only a further slight (not statistically significant) reduction.
Our results indicate that AE, differently from CSC, does not induce an inflammatory response by EC and HM.

TRATTAMENTO FARMACOLOGICO E ADERENZA IN SOGGETTI <40 ANNI CON DIAGNOSI DI IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE IN LOMBARDIA

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Contesto. L'ipercolesterolemia familiare è una malattia autosomica co-dominante, che aumenta marcatamente la concentrazione delle lipoproteine a bassa densità nel plasma, provocando malattia aterosclerotica e coronarica prematura. Le statine sono attualmente la terapia farmacologica di prima scelta e sono raccomandate a partire dall'età di 8 anni. Obiettivo di questo studio è descrivere l'approccio terapeutico in pazienti con diagnosi di FH eterozigote, i cambi di terapia e l'aderenza al trattamento.
Metodi. Dai database amministrativi della Regione Lombardia, sono stati identificati gli individui che avevano ricevuto esenzione per ipercolesterolemia familiare eterozigote (HeFH, codice 025.272.0) nel periodo 1/1/2003-31/12/2011 e che avessero meno di 40 anni alla data di rilascio dell'esenzione. Tra questi, sono stati selezionati i pazienti con prima prescrizione di statina (wash out 3 anni) da 2 mesi prima a 6 mesi dopo la data di esenzione e almeno un anno di follow-up. Questi soggetti sono stati caratterizzati per prima statina prescritta, potenza della prima statina prescritta e numero o tipologia di cambi nel primo anno di trattamento. L'aderenza a un anno è stata calcolata come PDC (proportion of days covered).
Risultati. I criteri di selezione hanno identificato 1404 pazienti, di età media±deviazione standard 33,3±5,4 anni, di cui il 62% maschi. Il 33,4% ha iniziato il trattamento con simvastatina, il 29,6% con rosuvastatina e il 21,6% con atorvastatina; il 4,6% ha ricevuto come la prescrizione iniziale la combinazione simvastatina+ezetimibe. Complessivamente, il 13,9% della coorte è stato inizialmente trattato con una statina ad alta potenza. L'approccio terapeutico iniziale non mostrava differenze rilevanti per sesso, né per età, tranne che per una lieve tendenza a prescrivere inizialmente alle donne statine a bassa potenza. Il 23,4% della coorte ha effettuato almeno un cambio di principio attivo e/o di dosaggio entro il primo anno; il 4,9% ha effettuato due cambi, il 2,1% tre o più cambi. In media, il primo cambio è avvenuto 4,6±3,2 mesi dopo la data di esenzione. Il cambio era più frequente in chi iniziava la terapia con pravastatina (35,2%) e meno frequente per chi iniziava con simvastatina (20,5%); inoltre, avveniva più spesso tra chi iniziava con una statina a bassa (28,0%) o ad alta (29,7%) potenza (rispetto a potenza intermedia, 20,3%). Non c'erano differenze per sesso, ma si osservava una leggera tendenza a una riduzione della percentuale di soggetti con almeno un cambio all'aumentare dell'età. I cambi riguardavano nel 34,5% una riduzione della potenza e nel 46,0% un aumento della potenza. Il 9,2% dei soggetti ha abbandonato la terapia dopo la prima prescrizione, l'8,4% dopo due prescrizioni. Complessivamente, la percentuale di pazienti con una copertura del trattamento (PDC) inferiore al 40% era pari al 23,5%, mentre solo il 32,6% risul-

tava coperto per almeno l'80% dei giorni. In media, la PDC era pari al 61,6%±26,3%, più alta per gli uomini (63,1% vs 59,2%, p=0,0064), per chi ha iniziato con atorvastatina (66,0%, p<0,001) e per chi ha effettuato almeno un cambio (68,9% vs 59,4%, p<0,001). L'aderenza media mostra un trend temporale positivo, aumentando da 51,2% nei pazienti che hanno ricevuto l'esenzione nel 2003 al 68,8% nei pazienti con esenzione nel 2011 (p<0,001).
Conclusioni. L'analisi di questa coorte di pazienti <40 anni con esenzione per HeFH ha mostrato che l'approccio terapeutico predilige simvastatina, atorvastatina e rosuvastatina. Il cambio di terapia è abbastanza frequente, e riflette probabilmente la necessità di aggiustare la terapia per evidenze di intolleranza o di scarsa efficacia, rispettivamente passando a statine a minore potenza nell'8% della coorte o a maggiore potenza nell11%. L'aderenza, nonostante il miglioramento nel tempo, resta complessivamente inadeguata anche in questa popolazione a rischio cardiovascolare particolarmente alto, con una proporzione elevata di pazienti che interrompe il trattamento dopo poche prescrizioni.

PROGENITOR ENDOTHELIAL CELLS AND ENDOTHELIAL MATURE CELLS IN SYSTEMIC SCLEROSIS: POSSIBLE MARKERS OF ENDOTHELIAL DAMAGE AND REGENERATION IN THE EARLY STAGES OF CONNECTIVE DISEASE

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Background. Systemic sclerosis (SSc,) is a chronic, multisystemic connective tissue disorder affecting the skin and various internal organs. To date, increasing evidence indicates that vascular damage is a primary event in the pathogenesis of SSc. Pulmonary hypertension (PH), a haemodynamic condition characterized by a mean pulmonary pressure ≥25 mmHg, is frequent in patients with SSc, representing one of the main cause of death.
Aim. To evaluate, in a group of SSc patients without evidence of resting PH, endothelial (EPCs) and circulating (CPCs) progenitor cells and circulating endothelial cells (CECs) number, in relation to organ involvement and to the changes in hemodynamics of the pulmonary circulation induced by physical effort detected by non-invasive ultrasound assessment of pulmonary artery pressures.
Methods. Forty-seven SSc patients (43F/4M; mean age 59.67±12.64 years) were subjected to physical stress ecocardiography and to a blood withdrawl at baseline (T0) and after the physical effort (T1) with the evaluation of CPCs, EPCs and CECs. CPCs were defined as CD34+/CD45dim, EPCs were defined as CD34+CD133+KDR+ and CECs as CD146+CD31+CD61+CD45-.
Results. Patients with telangiectasias showed basal levels of CPCs significantly lower than patients without telangiectasia [343 (180-500) vs. 552 (160-806) cells/106 events p=0.002]. In addition, a higher number of baseline CECs was observed in patients with diffuse form with respect to patients with limited form [18 (0-40) vs. 6(0-56) cells/106 events] and in patients with pitting scar skin lesions compared to patients without pitting scar [16(10-30) vs. 6 (0-56) cells/106 events, p=0.05]. CPCs, EPCs and CECs levels at T1 were measured in 40 patients and a significant reduction in the levels of CPCs was detected after exercise [520 (160-806) vs. 445 (124-1526) p=0.002]. A similar trend was observed also for EPCs and CECs, albeit not statistically significant. By analizing instrumental data, we found significant negative correlation between PAPs values detected at the peak of physical effort and EPCs levels at T1 (CD34+CD133+KDR+ and PAP r=-0.43 p=0.017). In addition, patients who showed a peak PAPs effort ≥50 mmHg showed lower EPCs levels at T1 [CD34+KDR+ 8(0-34) vs 12 (0-48) cells/106 events.
Conclusions. In SSc patients, the degree of clinical manifestations is linked to increased levels of endothelial cells index of endothelial damage such as CECs, and the concomitant reduction in cells expressing a regenerative potential. This potential pathogenic correlation is confirmed by changes in pulmonary pressure, which are strictly linked to the prognosis of these patients.

COME MIGLIORARE L'EFFICACIA IPOCOLESTEROLEMIZZANTE DI EZETIMIBE IN SOGGETTI STATINO-INTOLLERANTI NELLA PRATICA CLINICA: UNO STUDIO RETROSPETTIVO

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Obiettivo. Valutare la tollerabilità e l'efficacia di diversi approcci terapeutici per aumentare l'efficacia ipocolesterolemizzante di ezetimibe in pazienti statino-intolleranti.
Metodi. Abbiamo valutato retrospettivamente 3534 CRFs compilate per una prima visita nel periodo gennaio 2012-dicembre 2014 presso la Lipid Clinic dell'Università degli Studi di Bologna. Per questo studio abbiamo quindi selezionato 252 CRFs in base ai seguenti criteri: Ipercolesterolemia richiedente un trattamento farmacologico, Mialgie statine-correlate, Precedenti trattamenti interrotti con almeno 2 statine a basso dosaggio, Trattamento di base ben tollerato con Ezetimibe 10 mg 1 cp/die. Quindi, i seguenti trattamenti ipolipemizzanti sono stati aggiunti (in base al giudizio clinico del medico) al fine di migliorare l'efficacia ipocolesterolemizzante di Ezetimibe: Fenofibrato 145 mg, Rosuvastatina 5 mg 1 cp/settimana, Rosuvastatina 5 mg 2 cp/settimana, Riso rosso fermentato (standardizzato in Monakolina K 3 mg) + Berberine 500 mg, Berberina 500 mg b.i.d., Fitosteroli 900 mg + Fibra di psyllium 3,5 gr. I pazienti che hanno continuato a lamentare mialgie tollerabili sono stati poi trattati con coenzima Q10 in nanoemulsione (200 mg/die).
Risultati. Quasi il 50% dei pazienti trattati con Fenofibrato, Rosuvastatina 5 mg 2 cp/settimana, Riso rosso fermentato + Berberina 500 mg, 500 mg e Berberina b.i.d. associati ad Ezetimibe ha raggiunto il target di colesterolo LDL previsto per la specifica classe di rischio per malattia cardiovascolare, mentre la percentuale era molto più bassa per quelli trattati con Rosuvastatina 5 mg 1 cp/settimana o con Fitosteroli + Fibra di psyllium. L'11% dei pazienti trattati con Fenofibrato hanno dovuto cambiare ulteriormente terapia per ricomparsa di mialgie, mentre la percentuale è stata trascurabile con gli altri trattamenti testati. In pazienti con mialgia tollerabile residua (N. 52), il trattamento con coenzima Q10 per 8 settimane ha determinato un miglioramento medio dello score di mialgia percepita, passando da 4,8±1,9 a 2,9±1,3 (p=0,013), senza

differenze significative tra i diversi gruppi di farmaci ipolipemizzanti sottostanti, con scomparsa della mialgia in 18 soggetti su 52.
Conclusione. I pazienti che sospendono le statine per mialgia sono ampiamente eterogenei in termini di risposta a trattamenti alternativi, ma alcuni nutraceutici sembra essere efficaci e ben tollerati nel migliorare l'effetto di Ezetimibe sulla colesterolemia LDL. Il coenzima Q10 sembra migliorare la tollerabilità della mialgia residua in gran parte dei pazienti.

RIGIDITÀ ARTERIOSA E INDICI CLINICO-LABORATORISTICI DI EPATOPATIA STEATOSICA NON ALCOLICA (NAFLD) IN UN AMPIO CAMPIONE DI SOGGETTI NON TRATTATI FARMACOLOGICAMENTE: DATI DAL BRISIGHELLA HEART STUDY

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Razionale. L'associazione fra NAFLD e rischio cardiovascolare è ben nota. La diagnosi certa di NAFLD è bioptica, ma ai fini di screening in popolazione alcuni indici clinico-laboratoristici sono validati ed attendibili. Il nostro obiettivo è valutare se questi indici sono anche associati ad un diverso grado di rigidità aortica in un ampio campione di popolazione globalmente sana.
Metodi. Per questo studio abbiamo analizzato i dati clinico-laboratoristici e strumentali di 1731 soggetti adulti farmacologicamente non trattati (M: 49,9%, F: 50,1%; 57% non fumatori, 28% fumatori, 15% ex-fumatori; età media 58±15) visitati nel corso dell'ultimo survey di popolazione del Brisighella Heart Study. Quindi abbiamo valutato la correlazione fra pulse wave velocity (PWV), augmentation index (AI) e resistenze periferiche totali (TPR) [valutate tramite Vicorder®, Skidmore Medical Ltd, Bristol, UK, uno strumento validato per la stima della pressione arteriosa centrare e dei parametri correlati] e dei seguenti indici di NAFLD: Lipid Accumulation Product (LAP), Hepatic Steatosis Index (HSI), Fatty Liver Index (FLI). L'analisi multivariata ha incluso età, sesso, abitudine tabagica, attività fisica, PAS, PAD, frequenza cardiaca, LDL-C, HDL-C, TG, apoB, apoAI, acido urico siero e (in modo esclusivo) HSI, LAP o FLI.
Risultati. All'analisi univariate i 3 indici di NAFLD erano significativamente correlate fra loro ($p<0.001$), con PWV (tutti $p<0.001$), AI (tutti $p<0.001$) e TPR (LAP $p<0.001$; HSI e FLI $p<0.05$).
All'analisi multivariata, AI risultava meglio predetto da età (RR= 0.22, 95%CI 0.20-0.24, $p<0.001$), HSI (RR=0.22, 95%CI 0.16-0.28, $p<0.001$) ed apoB (RR=0.06, 95%CI 0.04-0.7, $p<0.001$).
PWV risultava meglio predetto da età (RR=0.43, 95%CI 0.04-0.06, $p<0.001$), HSI (RR=0.08, 95%CI 0.06-0.10, $p<0.001$) e PAS (RR=0.02, 95%CI 0.01-0.0, $p<0.001$).
TPR risultava meglio predetto da PAD (RR=0.19, 95%CI 0.01-0.02, $p<0.001$) e LAP (RR= 0.002, 95%CI 0.001-0.002, $p<0.001$).
Conclusione. In un ampio campione di popolazione diversi indici di NAFLD sembrano associate a markers di rigidità arteriosa. In particolare AI e PWV sono indipendentemente correlati ad HSI (più influenzato dai parametri antropometrici), mentre TPR a LAP (più influenzato dalla presenza di dislipidemia aterogena). Il valore prognostici di questa osservazione preliminare deve essere confermata su valutazioni longitudinali.

EFFECT OF AN INNOVATIVE PASTA ENRICHED WITH BIOACTIVE COMPONENTS AND FUNCTIONAL PROBIOTIC ON HDL CHOLESTEROL EFFLUX CAPACITY (CEC)

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Cholesterol efflux capacity (CEC) is the primary atheroprotective function of high density lipoproteins (HDL) and represents their ability to accept cholesterol from macrophages, the first step of reverse cholesterol transport. Cholesterol efflux to HDL mainly occurs through the activity of the membrane transporters Scavenger Receptor class B type I (SR-BI), ATP Binding Cassette A1 (ABCA1) and G1 (ABCG1), which is strictly dependent on HDL structure. Also the aqueous diffusion (AD) play an important role in this process. Interestingly, recent findings suggest that serum-HDL CEC, a metric of HDL functionality, may be considered a more relevant, predictive parameter than HDL-C concentrations for cardiovascular risk evaluation. Epidemiological evidence indicate that high consumption of whole grains is associated with a reduced risk of chronic diseases related to diet such as cardiovascular and metabolic disease. The aim of this study was to evaluate the effect of consumption of a new kind of whole-wheat flour with added β-glucan from barley and spores of B. coagulans on HDL functionality, measured as CEC. A radomized parallel study of 12 weeks duration has been conducted in 40 healthy volunteers. Recruited subjects were assigned to two treatments in randomized order:

1) Experimental pasta made with whole-wheat flour enriched in β-glucan from barley and spores of B. coagulans GBI-30, 6086.
2) Control pasta produced with the same technological process and with the same, but not integral, variety of wheat as the functional one.

CEC measurement was performed ex vivo on whole plasma collected from subjects before and after treatment with the innovative pasta or with the control one. The individual cholesterol efflux pathways were evaluated by using specific, widely accepted cell-based radio isotopic assays. AD - and ABCA1 - mediated CEC remained unchanged between treated and controls subjects and did not show to be affected by the treatment. Neverthless ABCG1-mediated CEC was increased in subjects treated with the innovative pasta. Additionally ABCG1-mediated CEC was found to be slightly improved in the group of treated subjects compared to the placebo group. On the base of these preliminary results, we observed that pasta treatment induces an increased in HDL-CEC mediated by ABCG1 transporter, which plays a fundamental role in cholesterol efflux process and which activity is importantly related to intracellular inflammatory pathways. Further investigations on serum-HDL CEC in treated subjects, as well as its correlation with clinical indeces of inflammation, would be useful to better define the protective effects of this kind of innovative pasta, expecially on cardiovascular disease.

IL PESO DEI FATTORI DI RISCHIO CARDIOVASCOLARE: INVECCHIAMENTO ED ATEROSCLEROSI NON SONO SEMPRE SINONIMI

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L'età è considerata uno dei principali fattori di rischio "non modificabili" per malattie cardiovascolari. Si tende pertanto a considerare l'aterosclerosi come una malattia dell'invecchiamento. Scopo: confrontare la prevalenza di ateromasia carotidea fra soggetti ultraottantenni con anamnesi negativa per vasculopatia ed eventi cardio-cerebrovascolari, e soggetti adulti ipertesi, valutando l'associazione con i principali fattori di rischio cardiovascolare (CV).
Centosettantanove pazienti: 69 ultraottantenni (età media: 88,5 anni) ricoverati presso la Clinica di Medicina Interna e Geriatria, e 110 adulti affetti da ipertensione arteriosa essenziale (età media: 53,3 anni), afferenti al nostro Centro Ipertensione.
La prevalenza di placca carotidea non risultava significativamente differente tra le due popolazioni studiate (51,8% negli adulti vs 58,0% negli ultraottantenni), nonostante gli oltre 35 anni di differenza nell'età media. In entrambe le popolazioni, la maggior parte delle placche era di tipo iperecogeno. Negli adulti ipertesi il fumo rappresentava il principale fattore di rischio CV associato alla presenza di placca carotidea (OR 2,41; p=0,024), mentre negli ultraottantenni il fattore di rischio maggiormente associato risultava l'ipertensione arteriosa (OR 10,5; p<0,001). Escludendo dagli ultraottantenni i soggetti ipertesi, la prevalenza di placca carotidea era significativamente superiore negli adulti (51,8% vs 27,6%; p=0,020).
I nostri risultati confermano l'importanza dei principali fattori di rischio CV, in particolare dell'ipertensione arteriosa, soprattutto nella popolazione ultraottantenne, dove la relazione appare essere ancora più evidente, probabilmente per la prolungata esposizione agli stessi. Invecchiamento non è quindi sinonimo di "inevitabile aterosclerosi" essendo invece i fattori di rischio nel loro insieme a determinare il "cattivo invecchiamento" dell'albero arterioso. Da qui l'importanza di agire fin dalla giovane età per prevenire l'insorgenza dei diversi fattori di rischio CV, trattandoli in maniera adeguata quando presenti, a qualsiasi età. Questa è probabilmente l'unica strada per evitare la «medicina delle conseguenze» con il fine di ottenere una longevità attiva.

ALTERAZIONI DEL PROFILO LIPIDICO IN UNA COORTE DI DIABETICI ANZIANI SARDI

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Obiettivi. Le alterazioni del metabolismo lipidico sono un importante fattore di mortalità e morbilità nel paziente diabetico, soprattutto se anziano (1, 2). Lo scopo del presente studio è stato quello di indagare l'eventuale relazione tra invecchiamento e parametri del profilo lipidico in una coorte di diabetici anziani di origine sarda.
Materiali e Metodi. Il reclutamento di 3.856 pazienti con diabete di tipo 2 afferenti all'Unità di Diabetologia dell'AOU di Sassari, è stato effettuato in base all'età distinguendo old (75-84 anni, di cui 763 M/1028F) e oldest old (≥85 anni, 799 M/1266 F). Sono state raccolte variabili quali: glicemia a digiuno, HbA1c, profilo lipidico (CT, HDL, TG e LDL), i rapporti CT/HDL e LDL/HDL. Il confronto è stato effettuato tra old e oldest old separatamente nei soggetti di sesso maschile e femminile.
Risultati. I soggetti old presentavano valori elevati di CT (M: 255±54, F: 271±56 mg/dL), TG (M: 241±79, F: 260±96 mg/dL), LDL (M: 166±34, F: 184±31 mg/dL) accanto a ridotti valori di HDL (M: 40±11, F: 35±14). Nei soggetti oldest old i valori del colesterolo erano pressochè nella norma nei maschi (199±41 mg/dL), ma non nelle femmine (225±43 mg/dL). In queste ultime i rapporti CT/HDL e LDL/HDL risultavano significativamente superiori rispetto alle donne old.
Conclusioni. Tra i soggetti anziani dislipidemici e diabetici sono state rilevate differenze di genere con valori significativamente superiori nelle donne old rispetto agli uomini di pari età. Tuttavia nei pazienti appartenenti alla fascia di età più avanzata (oldest old) la dislipidemia è assente o interessa esclusivamente la popolazione femminile. Ulteriori indagini saranno necessarie per scoprire le cause di tale disparità di genere.
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EFFETTI DI UN INTERVENTO NUTRIZIONALE SU UNA POPOLAZIONE FREE-LIVING: DATI DALLO STUDIO DI BRISIGHELLA

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Contesto. Nonostante gli avanzamenti della medicina, il carico di mortalità e morbidità delle malattie cardiovascolari sta aumentando in molti paesi. Ci sono evidenze che attribuiscono questa epidemia ad un'alimentazione scorretta.
Obiettivo. Valutare gli effetti concreti ed eventualmente riproducibili di un intervento di educazione nutrizionale nella popolazione free-living di Brisighella (RA). Se efficace questo costituirebbe un potentissimo strumento di prevenzione a basso costo per il Sistema Sanitario.
Metodi. Lo studio di Brisighella segue questa popolazione dal 1972 con controlli quadriennali. Visto l'alto rischio cardiovascolare si è deciso di iniziare una fase di intervento: fra il 1986 e il 1990 è stato attivato un centro di educazione nutrizionale con il compito di fornire indicazioni pratiche e materiale cartaceo per un'alimentazione più sana. Durante i controlli, oltre alla visita generale ed al prelievo ematico, venivano raccolti anche dettagliati diari alimentari. Noi abbiamo analizzato i dati del 1984 (693 maschi e 733 femmine) e del 1992 (788 maschi e 805 femmine), ovvero pre e post intervento, confrontando le variazioni dei marker di rischio cardiovascolare con le modifiche dell'alimentazione emerse dai diari, per valutarne le correlazioni.
Risultati. Abbiamo riscontrato un notevole miglioramento nelle abitudini alimentari, con una marcata diminuzione della quantità assunta di burro (-10,2 g/die, p<0,0001), strutto (-3 g/die, p<0,0001),

lipidi (-6 g/die, p<0,0001), colesterolo alimentare (-33,1 mg/die, p<0,0001), acidi grassi saturi (-8,3% sui grassi totali, p<0,0001) e parallelamente un aumento importante di fibre (+18 g/die, p<0,0001) e olio d'oliva (+9,6 g/die, p<0,0001). Ciò si è tradotto in una riduzione significativa dei parametri ematici: colesterolo totale (-34,3 g/dl, p<0,0001), trigliceridi (-54,1 g/dl p<0,0001) e LDL (-23,6 g/dl, p<0,0001). Anche la pressione arteriosa sistemica è calata (PAS -9,4 mmHg, p<0,0001 e PAD -5,5 mmHg, p<0,0001). Questi benefici si sono mantenuti nel tempo.
Conclusioni. Risulta indubbia l'efficacia e la riproducibilità di agire sull'alimentazione a livello di popolazione generale per ridurre notevolmente alcuni dei più importanti fattori di rischio cardiovascolare.

TRAINING REDUCES FLUORESCENT PLATELETS AND ERYTHROCYTE FRAGMENTS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

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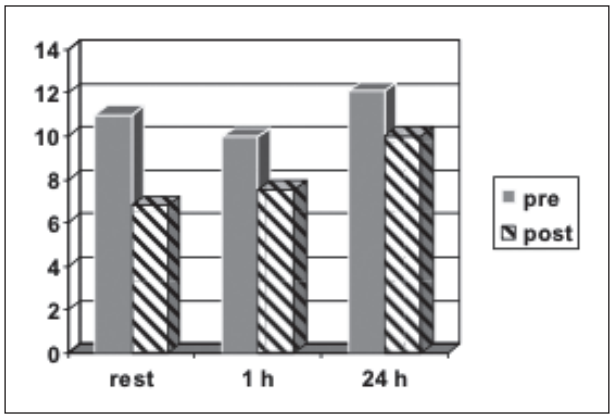
Background. Training is a documented effective treatment in patients affected from peripheral arterial disease (PAD). Platelet activation plays a pivotal role in atherosclerosis progression and cardiovascular events. Reticulated platelets (IPF) reflects activity of bone marrow , recently they have been associated to cardiovascular complications and atherosclerosis with unstable conditions (e.g. acute coronary syndrome). Presence of a width blood red cell distribution is considered recently as a prognostic factor for coronary artery disease, a high RDW value depends greatly on presence of red blood cells fragmentation (FRC); this parameter may depend on different conditions such as inflammation, and oxidation and is connected with different risk factors such as hypertension and diabetes. Few data can be found for patients with peripheral arterial disease on training. We aimed to evaluate the effects of aerobic training on IPF and FRC at rest and after maximal walking exercise before and after training.
Methods and Results. We enrolled 12 patients with intermittent claudication. They were submitted to a 15 days aerobic training period (cycling and treadmill exercise under maximal walking capacity). IPF, MPV, PLT count and FRC were analyzed at rest, 1 hour after maximal treadmill test and after 24 hours, these evaluations were performed at the beginning and at the end of the training period. The Lab parameters were analyzed with impedentiometry, fluorimetry (oxazyne) and optical methods (Sysmex Xn-1000, Sysmex Corporation, Kobe, Japan). Walking distance was measured with treadmill (3,2 km/h, 2-10% slope), maximal test was prolonged to the maximal tolerated claudication pain.
Platelets count was within normal range (216,9±40 109/l) and did not changed throughout the study; also MPV was unchanged (11,6±1,9 vs 11.45±0,8 fl) before and after the training; plateletcrit was slightly reduced (0,246±0,061 vs 0,282 ±0,018%). IPF count (*Figure*) slightly changed during maximal stress at the beginning of training with increase after 24 hours; after training the count decreased significantly (*p<0,05) at rest and 1 hour after, while it increased significantly after 24 hours (**p<0,05 vs rest ad vs 24 h-pre) but less than before training.
FRC decreased after triaining (0,381±0,121 vs 0,542±0,220%; p<0,05), maximal test slightly increased FRC after 1 hour, no significant change after 24 hours.
At the end of training, absolute walking distance increased (450±180 vs 250±108 m; p<0,05).



Discussion. Training reduces IPF in patients with peripheral arterial disease, IPF increase after acute maximal test and this phenomenon can be attenuated by training. We also observed a reduction in FRC. Presence of FRC in these patients may be caused by mechanical forces throughout a large surface of atherosclerotic plaques fragmenting red cells, ischemia reperfusion in claudication is another mechanism that can elicit formation of FRC and in addition high oxidative stress may contribute. IPF are associated with an increase platelets activity and a higher turnover; in this pathology both these condition can be found associated with oxidative stress, inflammation and endothelial dysfunction. Training improves oxidation, inflammation and endothelium function with favorable effects on platelets activation and turnover, furthermore these parameters may influence also FRC count.
Conclusion. Training in PAD patients reduces IPF and FRC with potential improvement in risk profile for atherosclerosis progression and reduction of cardiovascular events.

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BETA2GPI DRIVES TH1 INFLAMMATION IN ATHEROSCLEROTIC PLAQUES OF PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Background. Antiphospholipid syndrome (APS) is characterized by the presence of arterial and venous thrombosis, and by recurrent abortions, in patients with persistent presence of autoantibodies against phospholipid-binding proteins (aPL), such as beta2-Glycoprotein I (beta2GPI). Arterial thrombosis has been related to accelerated atherosclerosis in experimental models, however contrasting findings have been reported in clinical studies of patients with primary APS regarding an increased number of plaques or an abnormal arterial wall thickness.
Methods and Results. We investigated the cytokine production induced by beta2GPI, and its domains (DI, DII, DIII, DIV, DV) in activated T-cells that infiltrate in vivo atherosclerotic lesions of patients with primary APS atherothrombosis. We also examined the helper function of beta2GPI-specific T-cells for monocyte matrix metalloproteinase (MMP)-9 and tissue factor (TF) production, as well as their cytolytic potential and their helper function for antibody production. We report that APS patients with atherothrombosis harbor in vivo activated CD4+ T-cells that recognize beta2GPI in atherothrombotic lesions, and the majority of T-cells recognize their epitopes within the DI domain. beta2GPI and its domains induce T cell proliferation and expression of IFN-gamma in plaque-derived T-cell clones. beta2GPI-specific T-cells display helper function for monocyte MMP-9 and TF production, promote antibody production in autologous B cells, and express perforin-mediated and Fas-FasLigand mediated cytotoxicity.
Conclusions. Our data demonstrate that beta2GPI, and especially DI domain, drive a local Th1 inflammatory response, with subsequent plaque instability which eventually favors atherothrombosis. This finding explains the association between aPL and arterial thrombosis in spite of the lack of the evidence of surrogate markers for atherosclerosis in primary APS patients.

HOMOARGININE ADMINISTRATION REDUCES NEOINTIMAL HYPERPLASIA IN A RAT MODEL OF CAROTID ARTERY BALLOON INJURY

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Background. Homoarginine is an L-arginine homologue derived from lysine. Its physiological role is still in part unknown, but the structural similarity to L-arginine suggests that it may be an alternative substrate for enzymes that use L-arginine, such as nitric oxide synthase. Data from the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study have shown that low serum concentrations of homoarginine are an independent risk factor for all-cause and cardiovascular mortality. The strong association between low homoarginine levels and cardiovascular outcomes raises the question if this molecule could be relevant in the pathophysiology of vascular disease. Neointimal hyperplasia is an exaggerated inflammatory healing response after vascular injury. Of particular clinical interest is neointimal hyperplasia after balloon angioplasty and vascular stent deployment, since this may limit therapeutic success. Several studies have demonstrated that L-arginine supplementation is able to inhibit neointimal formation in experimental models of balloon injury.
Aim. The purpose of this study was to evaluate the effect of homoarginine on neointimal formation in a rat model of carotid artery balloon injury.
Methods. Thirty-six male Sprague-Dawley rats underwent endothelial injury at the level of the left carotid, followed by the insertion of a cannula into the right jugular vein. The cannula was connected to an osmotic infusion pump containing saline, L-arginine (30 mg/kg per day) or homoarginine (30 mg/kg per day). Fourteen days after balloon injury, blood was collected and left carotids were harvested for histological analyses. Systolic blood pressure was measured before and at the end of drug treatments.
Results. As expected, L-arginine administration significantly reduced the carotid intimal/medial area ratio compared to that of controls (0.69±0.40 vs 1.33±0.67, $p<0.05$). Homoarginine-treated rats also showed a significant reduction of the vessel intimal/medial area ratio versus controls (0.71±0.43 vs 1.33±0.67, $p<0.05$). No signs of toxicity and no changes in systolic blood pressure by treatment were detected among groups. Homoarginine serum concentrations were dramatically high only in homoarginine-treated rats (38.5±8.4 μM, 1.2±0.1 μM and 1.1±0.3 μM in homoarginine, L-arginine and control groups, respectively; $p<0.0001$). On the contrary, plasma L-arginine level was significantly increased in both L-arginine and homoarginine group compared to controls (137.3±15.6 μM,139.3±25.9 μM and 116.2±12.9 μM in L-arginine, homoarginine and control group, respectively; $p<0.05$). Moreover, in the homoarginine group a significant increase in serum concentration of ornithine was detected compared to control and L-arginine group (91.0±12.9 μM, 73.8±11.7 μM and 69.4±9.2 μM in homoarginine, L-arginine and control groups, respectively; $p<0.05$). Finally, L-arginine- and homoarginine-treated rats displayed higher nitrite levels compared to controls (2.4±1.2 μM, 2.2±0.8 μM and 1.1±0.4 μM in L-arginine, homoarginine and control groups, respectively; $p<0.05$).
Conclusions. Our study shows that *in vivo* homoarginine administration is able to inhibit neointimal formation in balloon-injured rat carotid arteries. Moreover, our data indicate that this effect could be due, at least in part, to an increased availability of L-arginine and nitric oxide production. Taken together, these evidences corroborate previous clinical data showing an association between homoarginine levels, endothelial function and cardiovascular health.

EFFECT OF HAZELNUT CONSUMPTION ON LIPID PROFILE, FATTY ACID COMPOSITION OF ERYTHROCYTE MEMBRANES AND OXIDATIVE STRESS MARKERS IN CHILDREN WITH PRIMARY DYSLIPIDEMIA

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Dyslipidemic patients are more prone to oxidative stress and inflammation, increasing their risk to develop atherosclerosis (1, 2). A dietary approach is crucial in the management of dyslipidemia, especially during childhood. Regular intake of nuts, source of mono- and polyunsaturated fatty acids, vitamins and bioactive compounds, could improve lipid profile and counteract oxidative stress (3).
The aim of the study was to investigate the effect of regular intake of hazelnuts consumed as a snack, on serum lipid levels, fatty acid composition of erythrocyte membranes and levels of DNA damage, as marker of oxidative stress, in children with primary dyslipidemia.
A randomized, controlled, parallel dietary intervention study was scheduled.
The study involved 60 children (11.5±2.5 years) affected by primary dyslipidemia, with total cholesterol and/or triglycerides value >95° percentile and BMI <95° percentile). Subjects received dietary guidelines and were divided into 3 groups of twenty subjects each, with the following conditions: one group consuming 0.43 g/kg of hazelnuts with skin, one group consuming 0.43 g/kg of hazelnuts without skin and a control group receiving only the dietary guidelines, without nuts, for 8 weeks. Before and after the intervention, blood samples were collected for the evaluation of serum lipid profile, fatty acid composition of erythrocyte membranes (by gas-chromatography), and levels of endogenous and oxidatively induced DNA damage in peripheral blood mononuclear cells (by comet assay).
Preliminary results on 20 subjects shown that hazelnut consumption significantly reduced the levels of total LDL-cholesterol (-6.37%, p=0.04) and H2O2-induced DNA damage (-26.5%, p=0.01), while increases the ratio HDL/LDL-cholesterol (+7.62%, p=0.01) and monounsaturated fatty acid composition of erythrocytes (+4.65%, p=0.006), as compared with control group. Further analysis of data on the whole group of subjects will help understanding the potential beneficial effect of hazelnut consumption on oxidative stress markers and lipid profile in children with dyslipidemia.

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IS FATTY LIVER AN INDEPENDENT RISK FACTOR FOR SUBCLINICAL ATHEROSCLEROSIS?

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Background. Non-Alcoholic Fatty Liver Disease (NAFLD) has been reported to be a risk factor for atherosclerosis. However, this association might be biased by the high prevalence of pro-atherogenic metabolic abnormalities in NAFLD. It has been reported that carriers of the M148M variant in the patatin like phospholipase 3 (PNPLA3) gene develop NAFLD without metabolic abnormalities.
Aims and Methods. To clarify whether fatty liver itself promotes vascular damage, we compared subclinical atherosclerosis in 3 groups:
1) subjects with NAFLD due to metabolic syndrome, but not carrying the PNPLA3 variant (metabolic NAFLD group) (n=89);
2) subjects with NAFLD due to the PNPLA3 M148M (genetic NAFLD) (n=31);
3) normal controls (n=16).

Fatty liver was demonstrated by ultrasounds. Carotid intima-media thickness (CIMT) was measured as marker of subclinical atherosclerosis. Comparisons were adjusted for age, gender and smoke by GLM.
Results. Age and gender were not different among groups. Subjects with metabolic NAFLD had significantly higher values of anthropometric variables, lipids, glucose and transaminases as compared to those with genetic NAFLD and controls (p<0.05). Overall, CIMT of subjects with metabolic NAFLD (0,85±0,18 mm) was significantly higher than that of subjects with genetic NAFLD (0,69±0,21 mm), which in turn was similar to controls (0,71±0,13 mm) [adjusted P=0.001]. These differences persisted when comparisons were performed according to the degree of liver steatosis.
Conclusions. We showed that subjects with metabolic NAFLD have increased subclinical vascular damage as compared to those with genetic NAFLD, thus suggesting that fatty liver per se might not be a risk factor for atherosclerosis.

RESPONSE TO AN ORAL FAT LOAD WITH A NON DAIRY CHEESE CREAM CONTAINING FERMENTED SOYBEAN EXTRACT SOY COMPARED TO A DAIRY CHEESE ON LIPID PROFILE

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Aim. To evaluate the response to an oral fat load (OFL) with a non dairy cheese cream containing fermented soybean extract soy, compared to a dairy cheese, on lipid profile in healthy subjects.

Methods. Oone hundred and twenty-four healthy subjects underwent an OFL performed using a mixture containing non dairy cheese cream, containing fermented soybean extract 75% (Valsoia Lo spalmabile®), or a placebo dairy cheese cream. We evaluated the variation of lipid profile and high-sensitivity C-reactive protein (Hs-CRP). Blood samples were drawn before and 3, 6, 9, and 12 h after the fat load.
Results. During the OFL, there was an increase of total cholesterol (TC), and triglycerides (Tg) at 3, 6, and 9 hours compared to time 0 with both placebo and active treatment; however, TC and Tg at 6 hours were lower with active treatment compared to placebo. Regarding low density lipoprotein-cholesterol (LDL-C), there was an increase at 3, 6, and 9 hours compared to time 0 during the OFL performed with placebo diary cheese, while during the OFL performed with the active treatment, there was an increase of LDL-C only at 6 hours; LDL-C value recorded at 6 hours with active treatment was lower than the one recorded at the same time with placebo. A decrease of high density lipoprotein-cholesterol (HDL-C) was recorded at 6, and 9 hours compared to time 0 with placebo, but not with the active treatment; moreover, HDL-C value recorded with active treatment was higher than the one observed with placebo at 6 and 9 hours. There was an increase of Hs-CRP at at 3, 6, and 9 hours compared to time 0 with placebo, while no differences were recorded with the active treatment. Hs-CRP value observed with active treatment was lower than the one observed with placebo at 6 hours.
Conclusions. A non dairy cheese cream, containing fermented soybean extract 75%, gave a minor increase of lipid profile and of Hs-CRP during OFL compared to a placebo dairy cheese cream in healthy subjects.

CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF INDIVIDUALS WITH LOW CHOLESTEROL SYNDROMES: A COMPARISON BETWEEN FAMILIAL HYPOBETALIPROTEINEMIA (FHBL) AND FAMILIAL COMBINED HYPOLIPIDEMIA (FHBL2)

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Background and Aim. The most frequent cause of low cholesterol are familial hypobetalipoproteinemia (FHBL), due to truncating or missense mutations in the APOB gene, and familial combined hypolipidemia (FHBL2), due to inactivating mutations in the ANGPTL3 gene. A direct comparison of lipid phenotypes of these two conditions has never been carried out. In addition, while an increased prevalence of liver steatosis in APOB-linked FHBL has been consistently reported, the hepatic consequences of FHBL2 appear to be less well established. Therefore, the purpose of the present study was to compare the clinical and biochemical characteristics of FHBL and FHBL2 individuals.
Methods. Clinical and biochemical characteristics as well as prevalence and degree of hepatic steatosis, evaluated by abdominal ultrasonography, was assessed in 119 FHBL subjects carrying mutations in APOB gene, 64 FHBL2 subjects carrying the ANGPTL3 S17X loss-of function mutation (6 homozygotes and 59 heterozygotes) and 210 normolipemic controls.
Results. As compared to controls, FHBL and FHBL2 individuals showed significantly reduced plasma concentrations of both LDL-C and total triglycerides (TG). However, a steady decrease of LDL-C was observed from heterozygous to homozygous FHBL2 to FHBL individuals (106.1±33.5 mg/dl vs 56.5±10.4 mg/dl vs 38.8±20.2 mg/dl respectively; p<0.001). Conversely, the lowest HDL-C levels was detected in homozygous FHBL2 (25.8±7.0 mg/dl). TG levels were comparably reduced in FHBL and homozygous FHBL2 (57.7±49.7 mg/dl vs 45.6±8.8 mg/dl), while heterozygous FHBL2 tended to have higher TG levels (102.4±50.7 mg/dl; p<0.001). Hepatic steatosis was observed in all FHBL individuals (100%), while its prevalence was comparable in FHBL2 and controls (45.3% vs 39.7%; p=0.5). Furthermore, FHBL individuals showed a more severe degree of hepatic steatosis as compared to the other groups. Comparison analysis performed according to the presence of hepatic steatosis in each group revealed that in FHBL2 individuals, but not in controls, the presence of fatty liver was independent of obesity, lipid profile and markers of insulin resistance.
Conclusions. Our results demonstrate that truncating mutations in APOB gene cause a more profound cholesterol-lowering effect compared to ANGPTL3 LOF mutations. Conversely, markedly reduced HDL-C levels was the unique lipid trait associated to inactivating mutations in the ANGPTL3 gene. In addition, FHBL was associated with higher prevalence and a more severe degree of hepatic steatosis. These findings suggest that defects in VLDL assembly are crucial for the occurrence of reduced concentration of apoB-containing lipoproteins and liver steatosis. On the other hand, the lack of association with fatty liver may be a distinctive characteristic of FHBL2 hypocholesterolemic syndrome.

RUOLO DELLA VARIANTE RS738409 DEL GENE PNPLA3 SU PARAMETRI METABOLICI E ANTROPOMETRICI IN UN CAMPIONE DI POPOLAZIONE ITALIANA SANA

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Introduzione e Obiettivi. Il gene PNPLA3 codifica per l'adiponutrina, proteina espressa prevalentemente a livello epatico e coinvolta nel metabolismo lipidico. Studi genomici di associazione hanno identificato il polimorfismo rs738409 come variante comune del gene PNPLA3 e come principale determinante genetico di accumulo di grasso epatico. La variante è un polimorfismo in singola base che determina una mutazione missenso con sostituzione di una isoleucina con una metionina in posizione 148. La frequenza dell'allele minore differisce tra le varie etnie ed è stata indagata nella popolazione italiana prevalentemente su casistiche di soggetti sovrappeso/obesi. È infatti dimostrata un'interazione gene-obesità nella determinazione del danno epatico e nell'espressione fenotipica della variante, che sembrerebbe alterare il metabolismo delle lipoproteine. Obiettivi di questo studio sono stati il calcolo della frequenza dell'allele minore e la valutazione della correlazione genotipo-fenotipo in un campione di popolazione italiana sana e normopeso.
Materiali e Metodi. Presso il Centro trasfusionale del Policlinico Umberto I di Roma sono stati arruolati 392 donatori di sangue a cui, previa firma del consenso informato, è stato richiesto di com-

pilare un questionario concernente dati anamnestici ed antropometrici. Le analisi ematochimiche ed l'analisi genetica sono state effettuate su campione di sangue prelevato in sede di donazione. La genotipizzazione è stata effettuata con metodo TaqMan.
Risultati. La casistica è risultata composta da soggetti di età media 41,5 anni, prevalentemente di sesso maschile (maschi 73%) e con IMC medio 24,7 kg/m^2. La frequenza dell'allele minore è risultata pari al 29%. Abbiamo rilevato valori di colesterolo totale, LDL e trigliceridi progressivamente più bassi negli eterozigoti e negli omozigoti per la variante rara, pur non sussistendo significatività statistica tra genotipi. Gli omozigoti M148M hanno mostrato rispetto ai wild type I148I valori di trigliceridi significativamente più bassi (TG I148I=86 mg/dL; TG M148M=70 mg/dL; p=0,038) ed un rischio inferiore di circa il 70% di presentare TG superiori alla media del campione (OR=0,331; IC 95% =0,110-0,994; p corretta per età e sesso =0,049).
Conclusioni. I nostri risultati mostrano che la variante è comune nella popolazione italiana sana e che è associata ad un profilo ipolipemico anche in soggetti normopeso. Ulteriori studi sono necessari per confermare questo dato.

A RARE APOC-III LOSS OF FUNCTION MUTATION IN A SUBJECT WITH PRIMARY HYPOBETALIPOPROTEINEMIA

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Background. Familial hypobetalipoproteinemia (FHBL) includes an heterogeneous group of co-dominant monogenic disorders characterized by reduced plasma levels of total cholesterol (TC), LDL-cholesterol (LDL-C) and apolipoprotein B (apoB). In 50% of the cases FHBL is due to loss of function (LOF) mutations in APOB gene (ApoBlinked FHBL); less frequently FHBL is due to LOF mutations in PCSK9 and ANGPTL3 genes. However in many subjects the gene responsible for the FHBL phenotype is unknown (Orphan-FHBL).
Methods. We applied next generation sequencing (NGS) technology to investigate the molecular bases of hypobetalipoproteinemia in a patient who did not carry mutations in the three major candidate genes (APOB, PCSK9 and ANGPTL3).
Results. The patient was a 38 years-old woman with the following lipid profile: CT 123 mg/dl, LDL-C 41 mg/dl, HDL-C 82 mg/dl, TG 30 mg/dl, ApoB 35 mg/dl, apoC-III 2 mg/dl. She had no hepatomegaly; AST and ALT were within the normal range. Physical examination was negative. Peripheral blood smear revealed the presence of acanthocytes. NGS showed that the patient was heterozygous for a nucleotide substitution in the APOC3 gene, located in the donor splice site of intron 2 [c.55+1G>A]. This mutation was predicted to disrupt the mRNA splicing leading to the skipping of exon 2 in mature mRNA. The joining of exon 1 to exon 3 is predicted to cause the formation of a premature termination codon. This mutation explains the low apoC-III level found in patient's plasma. This mutation, has been found to be associated with lower plasma TG, higher HDL-C and lower LDL-C in family as well as in population studies. The frequency of this mutant allele in European population is 0.2%.
Conclusions. This study suggests that subjects showing a plasma lipid profile compatible with the diagnosis of heterozygous FHBL may be carriers of LOF mutations of APOC3.

IDENTIFICATION OF TWO RARE VARIANTS IN LMF1 GENE IN A PATIENT WITH SEVERE HYPERTRIGLYCERIDEMIA

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Introduction. Severe Hypertriglyceridemia (HTG) is a rare disease characterized by levels of triglycerides (TG) higher than 11 mmol/L which are associated with eruptive xanthomas and pancreatitis. The disease is transmitted by an autosomal recessive inheritance and mutations in the Lipoprotein Lipase (LPL) or in Apolipoprotein A-V (APOA5) genes are the most frequent cause. A few number of cases have been ascribed to mutations in Apolipoprotein C-II (APOC2), Glycosyl-Phosphatidyl-Inositol-anchored HDL-Binding Protein (GPIHBP1), and Lipase Maturation Factor-1 (LMF1) genes.
Patient, Materials and Methods. The case report is based on a male patient aged 44 with TG levels of 15.9 mmol/L, normal cholesterol levels, eruptive xanthomas, hepatosplenomegaly and mild hepatic steatosis. One out of 5 sibling shows HTG. After treatment with low-fat diet, omega-3 fatty acids and fenofibrate the patient still shows high TG levels (10.1 mmol/L). The genetic screening included the direct sequencing of the coding region and the exon-intron junctions of the above genes and the identification of large rearrangements in the LPL gene by SALSA MLPA kit (MRC-Holland).
Results. Only common single nucleotide polymorphisms were found in LPL, APOA5, APOC2 and GPIHBP1 genes, whereas 2 very rare variants were identified in the LMF1 gene. The variant c.1351C>T leads to a missense substitution (p.Arg451Trp) previously identified in hypertriglyceridemic patients. The Minor Allele Frequency (MAF) is 0.0032 and in silico predictions support the pathogenic role of the variant (SIFT: damaging; Polyphen HumDiv and HumVar: Probably damaging). The second variant c.410C>T (p.Ser137Leu) was never associated with HTG phenotype but was previously found during the exome sequencing of the Exome Aggregation Consortium (ExAC) with a MAF=0.000027. In silico predictions indicate a pathogenic role of the variant (SIFT: damaging; Polyphen HumDiv and HumVar: Probably damaging).
Conclusions. The genetic causes of severe HTG could be ascribed to the presence of two rare variants in the LMF1 gene.

REPLACEMENT DIET WITH PRODUCTS MADE WITH ORGANIC KHORASAN WHEAT (KAMUT®) IN PATIENTS WITH DIABETES - A DOUBLE-BLIND DIETARY INTERVENTION TRIAL

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Background. An increasing interest in modifying the global risk profile of patients with type 2 diabetes mellitus through modification of dietary habits has been reported in recent years. Khorasan wheat (Kamut®) is an ancient grain with widely acclaimed beneficial effects on human health.
Aim. The aim of this study was to examine whether a replacement diet with products made with organic khorasan wheat could provide additive protective effects in reducing lipid, oxidative and inflammatory risk factors, in patients with type 2 diabetes mellitus in comparison to a similar replacement diet using products made from organic modern wheat.
Methods. We conducted a randomized, double-blinded cross-over trial with two intervention phases on 21 patients with a diagnosis of type 2 diabetes (14 females; 7 males). The participants were assigned to consume products (bread, pasta and crackers) made either from Kamut® or control semi-whole-grain wheat for 8 weeks in a random order. An 8-week washout period was implemented between the interventions. Laboratory analyses were performed both at the beginning and at the end of each intervention phase.
Results. At a general linear model for repeated measurements adjusted for age, sex, traditional risk factors, medication and diet quality, consumption of Kamut® products showed a significant reduction of metabolic risk factors such as total cholesterol (mean reduction: -7.0 mg/dL; -3.7%), low-density lipoprotein cholesterol (-3.8 mg/dL; -3.4%), blood glucose (-14 g/L; -9.1%) and insulin (-2.4 U/L; -16.3%). Similarly, there was a significant reduction in reactive oxygen species (ROS) and circulating levels of vascular endothelial growth factor. No significant differences from baseline in the same patients were observed after the conventional control wheat intervention phase. Moreover, the replacement diet with Kamut products resulted in a significant increase of serum magnesium (+5.3%).
Conclusion. The present results suggest that a replacement diet with Kamut® products helps patients with a diagnosis of type 2 diabetes to optimize their cardiovascular risk profile. This evidence could be of high clinical relevance for reducing the occurrence of a cardiovascular event in these patients.

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LOW DENSITY LIPOPROTEIN RECEPTOR/TRANSFERRIN CHIMERIC PROTEIN IMPROVES DYSLIPIDEMIA IN LOW DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT MICE

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Familial hypercholesterolemia (FH) is a well-characterized hyperlipidemia due, in most of the cases, to mutations in the LDL receptor (LDLR) gene; FH is characterized by elevated concentration of plasma LDL cholesterol (LDL-C) with consequent deposition of LDL-C in tendons, skin and arteries. Statins can lower cholesterol levels but are not always effective; patients unresponsive to this therapy have usually a poor prognosis. We have previously developed gene replacement strategies using PEGylated helper-dependent adenoviral (HD-Ad) vectors leading to hepatocyte-restricted expression of LDLR or VLDL receptor obtaining a complete rescue of the phenotype. Even though PEGylated vectors induced a milder innate response, intravenous administration can narrow the therapeutic window and reduce the clinical applicability of gene transfer. In order to further reduce host response, we have devised a therapeutic strategy for LDL reduction using a secreted chimeric protein. At this aim, we developed an HD-Ad vector for the expression of a secreted protein composed by the extracellular portion of the human LDLR fused in frame with transferrin (LDL-R/Tf). We have evaluated expression and function of LDL-R/Tf in cellular models as 293, COS and CHOldla7; subsequently, we have intravenously administered an HD-Ad vector expressing LDLR/TF in LDLR-deficient mice observing a significant reduction of total and LDL-C cholesterol. We plan to further evaluate the efficacy of the LDLR/TF chimeric proteins using alternative routes of administration in order to develop a safe gene transfer protocol more suitable for clinical applications.

ESECUZIONE DEL PROFILO LIPIDICO DI ROUTINE IN UN LABORATORIO CLINICO: IMPATTO SULL'IDENTIFICAZIONE DI FENOTIPI LIPIDICI ESTREMI

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Introduzione. Linee guida internazionali che utilizzano i parametri lipidici per la definizione del rischio cardiovascolare sono basate su studi epidemiologici che stabiliscono un legame tra le lipoproteine e la malattia cardiovascolare. Le linee guida ESC/EAS raccomandano che i soggetti asintomatici ad alto rischio di malattia cardiovascolare devono essere individuati e trattati con terapia ipocolesterolemizzante. Lo screening dei fattori di rischio, incluso il profilo lipidico, deve essere considerato nei maschi adulti con più di 40 anni e nelle femmine con più di 50 anni o in post-menopausa, particolarmente in presenza di altri fattori di rischio. La valutazione lipidica di base

suggerita comprende colesterolo totale, trigliceridi, LDL e HDL. L'utilizzo nei laboratori clinici di metodi diretti per la misurazione delle LDL e HDL ha nel tempo soppiantato i metodi precedenti basati sulla precipitazione chimica o sulla separazione delle sottoclassi.
Metodi. Sono state estratte dal database informatico del Laboratorio Analisi Chimico-Clinico e di Endocrinologia dell'ASMN di Reggio Emilia le determinazioni di colesterolo totale, trigliceridi, LDL, HDL e di Lp(a) effettuate nell'anno 2014. Le misurazioni dei parametri lipidici sono state effettuate con procedure automatizzate su analizzatori ADVIA® 1800 Chemistry System - Siemens. È stato utilizzato un metodo colorimetrico per la determinazione di colesterolo totale, trigliceridi, LDL, HDL e un metodo immunoturbidimetrico per la determinazione di Lp(a).
Risultati. Nell'anno 2014 sono state effettuate 131771 determinazioni di colesterolo totale (Media ± Deviazione Standard: 187±43 mg/dl), 128027 di trigliceridi (119±87 mg/dl), 102222 di HDL (55.5±16 mg/dl), 74329 di LDL (118±36 mg/dl), 1139 di Lp(a) (33±38 mg/dl). La percentuale di determinazioni di colesterolo totale >270 mg/dl è risultata pari al 2.4% (3614 determinazioni/3147 soggetti), trigliceridi >200 mg/dl pari al 9.7% (12491/9374), HDL ≤35 mg/dl pari all'8% (8234/6609), LDL ≥190 mg/dl pari al 3% (2270/2093), Lp(a) ≥50 mg/dl pari al 20.5% (234/209). Le determinazioni di LDL erano richieste nel 13.1% dei casi per soggetti ≤40 anni, nel 53.2% per soggetti tra 41 e 70 anni e nel 33.7% per soggetti ≥ di 71 anni. In particolare tra le LDL ≥190 mg/dl, l'89% delle richieste proveniva da medici del territorio rispetto all'11% dei reparti ospedalieri. Il 57.3% dei soggetti (1199) con LDL ≥190 mg/dl erano costituiti da femmine contro il 42.7% (894) di maschi; il 69.7% (1459) dei soggetti con LDL ≥190 mg/dl erano compresi nella fascia 41-70 anni.
Discussione. Il laboratorio clinico considerato è una struttura pubblica su base provinciale con un bacino d'utenza stimato pari a circa mezzo milione di utenti. L'elevato grado di automazione consente l'esecuzione di circa 6 milioni di esami/anno con 437500 determinazioni di parametri lipidici nell'anno 2014. Nelle linee guida integrate per la diagnosi ed il trattamento delle dislipidemie viene riconosciuto al laboratorio un ruolo fondamentale nell'allertare i pazienti e i medici circa la presenza di fenotipi lipidici estremi. Considerando l'Ipercolesterolemia Familiare (FH) come esempio e applicando i criteri del Dutch Clinic Lipid Network ai soli dati di laboratorio, nell'anno 2014 nella provincia considerata sono stati identificati 2093 soggetti con una diagnosi clinica di possibile/probabile FH (Dutch Score ≥3); in particolare 100 soggetti avevano LDL ≥250 mg/dl (Dutch Score ≥5). Solo l'1.6% (1232) delle richieste di LDL era riferito a ragazzi ≤18 anni, in contrasto con le linee guida integrate per la salute cardiovascolare e la riduzione del rischio in bambini e adolescenti che prevedono lo screening lipidico universale tra 9 e 11 anni. Nel referto di laboratorio sono stati inseriti dei commenti interpretativi a profili lipidici estremi (LDL >190 e >250 mg/dl) per facilitare il riconoscimento di eventuali soggetti FH: questo ha portato alla diagnosi molecolare di 25 soggetti con ipercolesterolemia familiare.

ASSOCIAZIONE TRA ipoHDL-COLESTEROLO ED IPERTRIGLICERIDEMIA IN RAPPORTO ALLO STATO DI GLUCOTOLLERANZA: DIFFERENZE TRA I DUE SESSI

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Introduzione. L'associazione di HDL-colesterolo basso e di ipertrigliceridemia, frequente nel diabete mellito, identifica pazienti con rischio aterogenico elevato.
Obiettivo dello studio. Abbiamo valutato in entrambi i sessi la prevalenza dell'associazione basso HDL-colesterolo con ipertrigliceridemia (ipoHDL-iperTG) in rapporto allo stato di glucotolleranza.
Materiali e Metodi. Abbiamo considerato 2402 soggetti (1064 M, 1338 F), afferenti all'Ambulatorio Ipertensione e Dismetabolismo della nostra Divisione. Dopo esecuzione di OGTT, 1202 soggetti sono risultati normoglucotolleranti (NGT), 926 prediabetici (IFG e/o IGT) e 274 diabetici (DM). Inoltre, dopo valutazione del profilo lipidico e considerando i riscontri di HDL-colesterolo <40 mg/dl nel maschio (50 nelle donne) e di trigliceridemia ≥150 mg/dl, 341 soggetti (193 M) presentavano ipoHDL-iperTG.
Risultati. Abbiamo evidenziato come la percentuale ipoHDL-iperTG aumenti significativamente con il peggiorare dello stato di glucotolleranza (9.8% nel gruppo NGT, 17.9% nei prediabetici, 20.8% nei DM) ($p<0.0001$). Considerando il sesso maschile abbiamo registrato un incremento significativo della percentuale di ipoHDL-iperTG nei prediabetici rispetto al gruppo NGT (17.6% vs 10.7%, $p<0.05$). Nel sesso femminile invece, indipendentemente dalla menopausa, abbiamo documentato progressivi e significativi incrementi della percentuale di ipoHDL-iperTG con il peggiorare della glucotolleranza (NGT = 9.2%, prediabetici =18.2%, DM=28.4%, $p<0.0001$). Considerando sia i pazienti NGT che i pazienti prediabetici, le percentuali di ipoHDL-iperTG tra i due sessi non erano significativamente diverse (negli NGT F=9.2% vs M=10.7%, nei prediabetici F=18.2%, vs M=17.6%). Nel gruppo dei pazienti con nuova diagnosi di diabete, invece, abbiamo riscontrato una prevalenza significativa della percentuale di ipoHDL-iperTG nel sesso femminile rispetto al maschile (28.4% vs 13.6%, $p<0.05$).
Conclusioni. Tali dati evidenziano come l'aterogenicità, espressa da ipoHDL-iperTG aumenti progressivamente, soprattutto nel sesso femminile, con il peggiorare dello stato di glucotolleranza.

TWO SNPS IN LDLR SHOW INCREASED FREQUENCY IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA WITHOUT MUTATIONS IN CAUSATIVE GENES

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Introduction. Familial Hypercholesterolemia (FH) is a dyslipidemia leading to high levels of LDL-cholesterol and to severe clinical signs including early atherosclerosis. FH is traditionally considered a monogenic disease due to mutations in LDLR, APOB, PCSK9 (dominant form) and in LDLRAP1 (recessive form). Different studies supported the idea that in a portion of FH patients without mutations in the above genes the disease cause could be polygenic, i.e., due to several Single Nucleotide Polymorphisms (SNPs) in different genes. We aim to verify if 4 SNPs in LDLR genes are associated with the disease in patients without mutations.
Materials and Methods. Among patients with clinical diagnosis of FH, we selected 90 unrelated patients without mutations in LDLR, APOB and PCSK9 (FH/M-). From sequencing data

of LDLR we retrieved the genotypes of 4 SNPs in LDLR previously associated with increased risk of cardiovascular disease: rs2228671 (c.81C>T; p.Cys27=), rs5929 (c.1617C>T; p.Pro539=), rs688 (c.1773C>T; p.Asn591=) and rs5925 (c.1959T>C; p.Val653=). We compared genotype, allele and haplotype frequencies between patients and 50 normolipidemic subjects.
Results. Genotype frequencies of rs688 and rs5925 are statistically different between FH/M- and controls (p=0.001 and p=0.013 respectively at the chi-square test). In particular the genotypes comprising the rare allele of both SNPs are more frequent in patients than in controls. The comparison of allele frequencies confirm that the rare allele is most frequent in patients than in controls with p=1.096E-11 for rs688 and p=0.001 for rs5925. The haplotype constituted by the rare allele of both SNPs is about 5 time more frequent in patients than in controls (0.520 vs 0.099; with p=2.7006E-12).
Conclusions. The SNPs rs688 and rs5925 in LDLR could be considered predisposing variants for the development of FH in patients without mutations in causative genes.

A CLINICAL STUDY: THE EFFICACY OF ALTERNATIVE INTERMITTENT LOW-DOSAGE TREATMENT STRATEGIES IN PATIENTS PREVIOUSLY INTOLERANT TO DAILY STATIN DOSAGE

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Background. Statin side-effects are considered to be contraindications to statin therapy, though they are not frequent.
Aim. Our aim is to examine the efficacy of alternative intermittent statin low-dosage regimens in patients with a previous intolerance to daily statin dosage.
Methods. We examined 41 patients (M:24; F:17). For each of them we elaborated a weekly cholesterol-lowering regimen, consisting in a daily lower medium statin dosage (equivalent to <5 mg rosuvastatin). Not to alter the molecules' pharmacokinetic profile, we have always prescribed subjects integral tablets. In a group of patients, ezetimibe was added to statin therapy so as to help them reach their LDL-C target. Survival analysis was carried out using Keplan-Meier method, in order to evaluate the long-term tolerance of an off-label individual treatment. A dependent T-test (P<0,05) was used to compare serum lipid profile, measured both at baseline and during the treatment, separately for each of the two cohorts (the statin and the ezetimibe-statin combination group).
Results. Between 56 off-label therapies elaborated, 11 (20%) were interrupted by patients. In this case, we had to reduce the previous therapy again. Kaplan-Meier survival analysis indicates that 75% of untolerated off-label treatment (n=8) had been interrupted after 81±40 weeks of treatment. On average, we prescribed the equivalent amount of 2,35±0,32 mg/day of rosuvastatin as monotherapy (n=18) and 1,92±0,85 mg/day of rosuvastatin plus 7,99±2,64 mg/day of ezetimibe as polytherapy (n=22). In the statin group, compared to the baseline level, we observed a relevant decrease in the total cholesterol (-87.5±34.9 mg/dL, -29%, n=17), LDL-C (-78.6±30 mg/dL, -38%, n=18) and non-HDL-C (-88.8±33 mg/dL, -36%, n=17) (all P<0.000001). Furthermore, in the ezetimibe-statin combination group, compared to the baseline level, we noticed a consistent decrease in TC (-78.5±52.1 mg/dL, -28%, n=21, P<0.00001), LDL-C (-75.2±52.8 mg/dL, -36%, n=19, P<0.00001) and non-HDL-C (-85.8±48.2 mg/dL, -35%, n=20, P<0.000001).
Conclusion. Some statin-intolerant patients can be successfully treated with low-daily statin dosages.

MEDIATORS OF ADIPOSE TISSUE-RELATED VASCULAR DISEASE RISK IN ADULT/OLDER POPULATION: THE ROLE OF RETINOL BINDING PROTEIN 4 (RBP4). A REPORT FROM PANGEA STUDY

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Introduction and Aim. Aging is a process characterized by the progressive deterioration of the ability to maintain homeostasis, resulting in an overall functional decline and increased risk of disease and death. The metabolic changes related to aging could lead to insulin resistance, a condition at the basis of the metabolic syndrome (MS). Aging is also characterized by a change in body composition, i.e. a reduction of subcutaneous fat in the face of an increase in visceral fat. Adipose tissue is a metabolically active organ and impaired secretion of adipokines is associated with insulin resistance, obesity, diabetes, atherosclerosis and cardiovascular disease. Retinol binding protien 4 (RBP4) is a recently identified adipokine correlated with obesity, insulin resistance and type 2 diabetes.
The aim of our study was to evaluate the correlation between plasmatic concentration of RBP4 and the cardiovascular risk in a cohort of adult elderly subjects.
Materials and Methods. 299 subjects aged 55-81 years, enrolled in the context of PANGeA study, completed a questionnaire on daily habits, health status and medical therapy. Every participant underwent clinical evaluation and a fasting blood sample was withdrawn for biochemical analysis including lipid profile, glycaemia, and plasma levels of RBP4. The global cardiovascular risk was assessed with the "Progetto Cuore" algorithm. For each subject we evaluated the presence of Metabolic Syndrome (MS), according to the NCEP-ATPIII criteria and we calculated a Metabolic Score from the sum of each component of MS.
Results. RBP4 concentrations were positively correlated with systolic (r0,21, P<0.001) and diastolic blood pressure (r 0.18 p 0.002), triglycerides (r 0:22, p<0.001), "Progetto Cuore" score (r 0:21, p<0.001) and Metabolic Score (r 0.27, p<0.001); while RBP4 concentrations negatively correlated with HDL-C (r-0.14, p 0.014). We did not detect any correlation between RBP4 and glucose, insulin, total and LDL-cholesterol.
By dividing the population into tertiles of "Progetto Cuore" score, as expected, a significant difference between the groups regarding age, BMI, SBP, DBP, insulin, glucose, triglycerides, HDL-c and waist circumference was observed. We also observed a significant increase in RBP4 levels going from the first to the third tertile (50.48±5.15 vs 59.32±7.18 mg/dl, p<0.01).
Similarly, subjects with MS showed higher values of BMI, SBP, DBP, insulin, glucose and triglycerides, compared to those without MS. Moreover RBP4 concentrations were significantly higher in patients with MS (59.71±1.17 vs 52.86±8.16 mg/dl, p 0.001).
The linear regression analysis showed that RBP4 is associated with cardiovascular risk calculated with "Progetto Cuore" score,

independently of triglycerides, waist circumference and Metabolic Score. Similarly, RBP4 concentrations are associated with Metabolic Score, independently of age or sex.
Conclusions. In our healthy adult/elderly population the RBP4 was found to be strongly associated with the overall cardiovascular risk, calculated with the "Progetto Cuore" algorithm, and the presence of MS, regardless other "traditional" risk factors. In particular, the RBP4 is directly associated with triglycerides and inversely to the c- HDL, supporting a role of this protein in lipid metabolism. The lack of an association between RBP4 and insulin resistance suggests that the association between RBP4 and MS is mediated, at least in part, by mechanisms different from insulin sensitivity. It will be interesting to collect follow-up data to assess the possible use of RBP4 in cardiovascular risk stratification in clinical practice.

FAMILY HISTORY AND CARDIOVASCULAR DISEASE (CVD) RISK FACTORS IN A POPULATION OF CHILDREN WITH HYPERCHOLESTEROLEMIA

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Aim. To determine the CVD risk profile and family history for premature CVD in children with hypercholesterolemia at the first access to our Lipid Clinic and and to estimate the prevalence of genetically-confirmed heFH.
Methods. 186 severely hypercholesterolemic children (median age 8.7 y, 80 male/106 female), at their first access to our Lipid Clinic, with positive family history for hypercholesterolemia and/or premature CVD and no secondary causes of hypercholesterolemia, were evaluated for: anthropometric measures, twelve-hour-fasting blood sample for total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and Triglycerides (Try) by enzimatic method; Lipoprotein(a)-Lp(a) levels by nephelometry; apolipoprotein B (apoB) and apoliporotein A1 (apoA1) by immunoturbidimetric assay and genetic analysis for LDL-receptor gene by polymerase-chain-reaction. None was on pharmacological treatment.
Statistics. Student's t test or Mann-Whitney test for independent samples.
Results. The 186 patients were divided in two groups: 90 (48%) have a family history for premature CVD in first and second degree relatives (CVD+), 96 (52%) only for hypercholesterolemia (CVD-). The prevalence of obesity (according to Cole) was 11.1% vs 11.46% (as the median values for italian children population) while the prevalence of overweight was 16.7% vs 27.08% (below the median), in the CVD+group. The lipid profile (mg/dl, mean±ds) in the CVD+group and CVD- was, respectively: TC 243.01±55.06 vs 223.96±53.19 (p=0.011), LDL-C 171.17±54.86 vs 147.51±53.39 (p<0.001), HDL-C 54.29±12.49 vs 58.46±14.36 (p=0.036), Try 78.26±42.60 vs 80.60±48.60 (p=0.761), apoB 112.58±32.88 vs 100.49±32.75 (p<0.005), apoB/apoA1 0.87±0.34 vs 0.75±0.35 (p=0.039), Lp(a) 26±31 vs 25.20±30.44 (p=0.125); 36 (40%) vs 21 (21.9%) had a mutation on the LDL-receptor gene (heFH).
Conclusions. We found that children with family history for premature CVD (CVD+) have a worse CVD risk profile (more elevated TC, LDL-C, apoB levels and apoB/A1 0.87) and 40% have genetically confirmed familial hypercholesterolemia; an accurate anamnestic detection and early determination of lipid profile and genetic analysis are very important in childhood to detect severely-hypercholesterolemic children and for beginning early CVD prevention.

A SINGLE INFUSION OF TRIMERIC APOA-I IN HYPERCHOLESTEROLEMIC RABBITS STABILIZES ATHEROSCLEROTIC PLAQUES AND INCREASES PLASMA CHOLESTEROL EFFLUX CAPACITY

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Introduction. Experimental and clinical studies have shown that intravenous administration of synthetic HDL (sHDL) containing human apoA-I is effective in inducing atherosclerosis regression. However, one main drawback of this therapeutic approach may be a rapid apoA-I turnover. To circumvent this problem, a recombinant high-molecular mass variant of human apoA-I, named Tetranectin-apoA-I, has been engineered by fusing three apoA-I molecules with the trimerization domain of human. This trimeric apoA-I does not pass the glomerular filters and hence shows a prolonged half-life as compared to normal apoA-I. Aim of the present study was to evaluate the effect of Tetranectin-apoA-I infusion on atherosclerosis in a rabbit model, widely used to test the efficacy of sHDL.
Methods. The study was performed on 18 male New Zealand white rabbits. To induce lipid-rich plaque formation, common carotid arteries were perivascularly injured and, starting from the day of the surgery, all animals were fed a 1.5% cholesterol diet for the whole duration of the study. 90 days after lesion induction, rabbits were randomly divided into 2 groups and i.v. treated, for one time, with 200 mg/kg of sHDL containing Tetranectin-apoA-I (TN-sHDL) or with placebo. All animals were fasted over-night and blood samples were collected before and at different time points after the end of the infusion for biochemical evaluations. Plaque changes were analyzed in vivo by intravascular intrasound (IVUS), performed before and three days after the treatment, i.e. at sacrifice. Animals were then sacrificed and carotids were harvested for histological analyses. Aqueous diffusion (AD) - dependent - and total-cholesterol efflux capacity (CEC) of rabbit plasma, collected before infusion, and at 4h and 72h after the end of the treatment, were evaluated using J774 murine macrophages.
Results. Total atheroma volume in the placebo group increased in the time between the first and the second IVUS evaluation (+7.09±2.33% from baseline). On the contrary a slight regression was observed vs baseline in TN-sHDL treated group (-0.35±1.97%, p<0.0001 vs placebo). At the maximum plaque burden, TN-sHDL treated rabbits displayed a significant lower macrophage content compared to that found in the placebo group (69.5±13.4% vs 84.3±9.3%, p<0.05). Starting from 2 min after the end of the infusion and up to 24 h, a significant increase in plasma free cholesterol was observed in rabbits treated with TN-sHDL (p<0.05 vs placebo). Moreover, four hours after the end of the infusion both AD-dependent- and total-CEC of TN-sHDL-plasma were significantly increased compared to that of placebo (3.35±0.56% vs 1.68±0.08% for AD-dependent-CEC, p<0.005, and 9.02±0.61% vs 4.43±0.58%, for total-CEC, p<0.00001).
Conclusions. Taken together our results demonstrate that a single intravenous infusion of TN-sHDL is able to inhibit carotid

plaque progression in hypercholesterolemic rabbits. This effect was associated with a reduction in plaque macrophage content, and a rise in both CEC and free cholesterol plasma concentration. The present data indicate that administration of TN-sHDL could potentially be a pharmacological approach for rapid plaque stabilization.

EFFICACIA DI UNA COMBINAZIONE DI NUTRACEUTICI SULL'ASSETTO LIPIDICO IN PAZIENTI AFFETTI DA SINDROME METABOLICA

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Introduzione. L'utilizzo di nutraceutici rappresenta una nuova modalità terapeutica nella gestione di alcuni fattori di rischio cardiovascolare, in particolare nel contesto della Sindrome Metabolica (SM). Scopo di questo studio era di valutare i potenziali effetti benefici dell'Armolipid Plus (ARMP, berberina 500 mg, lievito rosso 200 mg e policosanolo 10 mg), sull'assetto lipidico e in particolare sulle componenti maggiormente aterogene (LDL piccole e dense), in soggetti affetti da SM.
Materiali e Metodi. In questo studio multicentrico, condotto in doppio cieco, controllato verso placebo, 158 soggetti, di età compresa tra 28 e 76 anni, stabilmente in terapia da almeno 3 mesi sono stati randomizzati a ricevere ARMP (n=79) o placebo (n=79) per 24 settimane. Sono stati valutati i principali parametri antropometrici e vitali, colesterolemia totale (C-tot), LDL, HDL, trigliceridemia, colesterolo non HDL (NHDL-C) e LDL piccole e dense (LDL-pd).
Risultati. Al termine del trattamento, l'analisi effettuata sui 141 soggetti valutabili, 71 trattati con ARMP e 70 con placebo (PL), ha evidenziato un significativo miglioramento dell'assetto lipidico nel gruppo trattato con ARMP, con riduzione del C-tot (-13.2 mg/dl), LDL (-13.9 mg/dl) e NHDL-C (-15.3 mg/dl) ed aumento dell'HDL (+2.0 mg/dl). Tali variazioni risultavano altrettanto significative nel confronto vs placebo (C-tot: ARMP -13.2 mg/dl vs PL +2.7 mg/dl; LDL: ARMP -13.9 mg/dl vs PL +1.5 mg/dl; NHDL-C: ARMP -15.3 mg/dl vs PL +2.8 mg/dl, HDL: ARMP +2.0 mg/dl vs PL -0.1 mg/dl).
Nel campione di popolazione è stato identificato un sottogruppo di 104 soggetti con LDL-pd presenti (54 trattati con ARMP e 50 con PL) in cui le differenze determinate dal trattamento erano maggiormente evidenti. Inoltre, risultava significativo l'aumento di dimensioni delle LDL-pd (ARMP +1.04 Å).
Conclusioni. I risultati di questo studio dimostrano che, in una popolazione di soggetti affetti da SM, il trattamento con ARMP migliora l'assetto lipidico ed i fattori maggiormente aterogeni, riducendo così il rischio di sviluppo e progressione dell'aterosclerosi, particolarmente in soggetti con elevato rischio aterogeno, per la presenza di LDL-pd.

RUOLO DEI LIVELLI PLASMATICI DI MIP-1BETA ED EOTAXINA-3 IN PAZIENTI CON OBESITÀ D'ALTO GRADO

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Introduzione. L'obesità si associa ad un'alterata funzione endocrina del tessuto adiposo, caratterizzata da un'aumentata secrezione di adipochine proinfiammatorie. Numerose evidenze suggeriscono che tali sostanze, contribuendo all'insorgenza di uno stato infiammatorio cronico sistemico, possano essere direttamente coinvolte nello sviluppo delle complicanze metaboliche e cardiovascolari tipiche del paziente obeso. Obiettivo del nostro studio è stato quello di valutare, in pazienti affetti da obesità d'alto grado, l'effetto della chirurgia bariatrica nella regolazione dei livelli plasmatici di MIP-1β ed Eotaxina-3, recentemente identificate come nuove adipochine, e l'eventuale correlazione tra questi e i principali parametri antropometrici, biochimici e clinici.
Metodi. Nello studio sono stati arruolati 80 pazienti obesi, 25 maschi ed 55 femmine, con Indice di massa corporea (BMI) medio di 44,6±7,27 kg/m^2, in lista pre-operatoria per l'intervento di chirurgia bariatrica. I pazienti sono stati sottoposti a: rilevazione dei principali indici antropometrici, determinazione dei livelli plasmatici di MIP-1β ed Eotaxina-3, Leptina e Adiponectina, indagini biochimiche e strumentali per l'inquadramento metabolico e cardiovascolare. Per ciascun paziente, i livelli plasmatici di MIP-1β ed Eotaxina-3 sono stati nuovamente valutati a distanza di 10-12 mesi dall'intervento.
Risultati. Nella valutazione pre intervento i livelli plasmatici di MIP-1β presentano all'analisi univariata una correlazione positiva con lo spessore medio-intimale carotideo (IMT) destro (r=0,295; p=0,01) e sinistro (r=+0,268; p=0,02), BMI (r=+0,217; p=0,05), età (r=+0,235; p=0,037) e Leptina (r=+0,470; p=0,02), negativa con Adiponectina (r= -0,453; p= 0,03); i livelli di Eotaxina-3 sono maggiori nei pazienti di sesso maschile e presentano una correlazione positiva con colesterolo LDL (r=+0,229; p=0,04), Apolipoprotreina B (r=+0,297; p=0,012) e Leptina (r=+0,412; p=0,04). Nella rivalutazione post chirurgia bariatrica, i pazienti presentano una significativa riduzione dei livelli plasmatici di entrambe le adipochine (p=0,000).
Conclusioni. La stretta correlazione tra i livelli plasmatici di MIP-1β ed Eotaxina-3 ed i principali markers e fattori di rischio per l'aterosclerosi suggerisce un possibile coinvolgimento di entrambe le adipochine nella patogenesi dei disordini cardiovascolari associati all'obesità. La riduzione dei livelli sistemici di MIP-1β ed Eotaxina-3 potrebbe dunque rappresentare uno dei meccanismi attraverso cui l'intervento di chirurgia bariatrica riduce l'infiammazione e il rischio cardiovascolare negli individui gravemente obesi.

VALUTAZIONE ECOGRAFICA DEL TENDINE DI ACHILLE NELL'IPERCOLESTEROLEMIA FAMILIARE

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Obiettivi. Verificare la validità dell'ecografia del tendine di Achille per discriminare i pazienti affetti da Ipercolesterolemia Familiare (FH) nei confronti di altre dislipidemie; definirne il significato in rapporto ai livelli di colesterolemia e alle manifestazioni cardiovascolari precoci (CHD).
Pazienti e Metodi. Sono stati arruolati 98 soggetti: 33 pazienti affetti da FH eterozigote (geneticamente documentata); 31 pazienti con altre forme di dislipidemia (Ipercolesterolemia poligenica, Iperlipemia combinata, Sindrome Metabolica); 34 controlli normolipemici. Ciascun paziente è stato sottoposto a studio ecografico bilaterale del tendine di Achille: è stata valutata l'ecostruttura, lo spessore tendineo misurato a 2 cm dall'inserzione calcaneare e l'eventuale presenza di aree diffusamente disomogenee e/o ipo-anecogene caratteristiche di Xantomatosi (Achilles Tendon Xanthoma, ATX). Valori di spessore tendineo superiori o uguali ai cut-off sesso ed età specifici riportati in letteratura sono stati considerati indicativi di ispessimento tendineo (Achilles Tendon Thickening, ATT)).
Risultati. Il riscontro ecografico di ATT ha mostrato una elevata sensibilità (78.8%) per la diagnosi di FH, a fronte di una modesta specificità (51.6%). Utilizzando cut-off superiori, desunti nella popolazione arruolata, la specificità è incrementata al 77.4%, a fronte di una trascurabile riduzione di sensibilità (75.8%). Lo spessore del tendine di Achille è apparso correlato positivamente con i livelli di colesterolemia. Solo nel gruppo di soggetti FH è stata evidenziata la presenza di ATX (sensibilità 36.4% e specificità 100%). L'ecografia è apparsa 9 volte più sensibile dell'esame obiettivo nell'individuare ispessimento tendineo e 4 volte superiore nell'individuare xantomatosi e quest'ultima è risultata correlata con la storia di cardiopatia ischemica precoce.
Conclusioni. L'ecografia del tendine Achilleo risulta utile per un adeguato inquadramento e stratificazione del rischio nel soggetto ipercolesterolemico. Appare più sensibile rispetto all'esame clinico nell'identificare la presenza di ispessimento o xantomatosi, è facilmente applicabile nella pratica ambulatoriale ed è auspicabile possa essere implementata in un algoritmo diagnostico.

IDENTIFICATION AND CHARACTERIZATION OF A LARGE DELETION INCLUDING THE LIPOPROTEIN LIPASE GENE IN A PATIENT WITH SEVERE HYPETRYGLICERIDEMIA

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Introduction. Familial Severe Hypertriglyceridemia (HTG) is a rare autosomal recessive disorder characterized by high plasma levels of triglycerides (TG>10 mmol/L), eruptive xanthomas, lipaemia retinalis and recurrent pancreatitis, with a population prevalence of approximately one in a million. HTG is caused by mutations in five different genes: Lipoprotein lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II (APOC2), Glycosyl-phosphatidyl-inositol-anchored HDL-binding protein (GPIHBP1), and Lipase maturation factor-1 (LMF1).
Patient, Materials and Methods. A 32 years old woman with a diagnosis of severe HTG (TG>20 mmol/L) and several episodes of pancreatitis was recruited at Department of Clinical Medicine and Surgery, University of Naples Federico II. Following a therapy with Gemfibrozil and Omega 3, the patient still shows TG levels >6mmol/L. After genomic DNA extraction from peripheral blood samples, the 5 genes associated with HTG, were amplified by PCR and directly sequenced. SALSA MLPA kit (MRC-Holland) was used to identify large rearrangements in the LPL gene and the array-Comparative Genomic Hybridization (array CGH) was used to define the deletion boundaries.
Results. The patient is a compound heterozygote for 2 mutations in the LPL gene: c.755T>C (p.Ile252Thr) in the exon 5 and a large deletion of entire gene. The c.755T>C mutation was previously described as causative of lipoprotein lipase deficiency, while the second mutation leads to the absence of protein. For the first time, the deletion of the entire gene was better defined by array CGH indicating a deletion of 650 Kbp including four genes: SH2D4A, CSGALNACT1, INTS10, LPL. By a cascade screening, the patient's brother with maximum TG of 15.4 mmol/L, without previous pancreatitis, was analyzed and resulted to be a compound heterozygote carrying the same mutations of his sister.
Conclusions. The genetic analysis confirmed the diagnosis of severe HTG and allowed to identify and treat a relative before the development of acute pancreatitis.

A NOVEL CREB3L3 NONSENSE MUTATION IN A FAMILY WITH DOMINANT HYPERTRIGLYCERIDEMIA

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Objective. cAMP responsive element binding protein 3-like 3 (CREB3L3) is a novel candidate gene for dominant hypertriglyceridemia. To date only four kindred with dominant hypertriglyceridemia have been found to be carriers of two nonsense mutations in CREB3L3 gene (245fs and W46X). We investigated a family in which hypertriglyceridemia displayed an autosomal dominant pattern of inheritance.
Approach and Results. The proband was a 49 year-old woman with high plasma triglycerides (up to 1300 mg/dl; 14.68 mmol/l). Her father had a history of moderate hypertriglyceridemia and her 51 years old brother had triglycerides levels as high as 1600 mg/dl (18.06 mmol/l).
To identify the causal mutation in this family we analyzed the candidate genes of recessive and dominant forms of primary hypertriglyceridemia by direct sequencing. The sequencing of CREB3L3 gene led to the discovery of a novel minute frame shift mutation in exon 3 of CREB3L3 gene, predicted to result in the formation of a truncated protein devoid of function (c.359delG - p.K120fsX20). Heterozygosity for the c.359delG mutation resulted in a severe phenotype in the proband and her brother with a late in life expression and a good response to diet and a hypotriglyceridemic treatment. The same mutation was detected in the 13 years old daughter who to date is normotriglyceridemic.
Conclusions. We have identified a novel pathogenic mutation in CREB3L3 gene in a family with dominant hypertriglyceridemia with a variable pattern of penetrance.

TRANSGENIC MOUSE MODELS TO INVESTIGATE THE ROLE OF THE HIGH DENSITY LIPOPROTEINS (HDL) BOUND SPHINGOSINE-1-PHOSPHATE (S1P) IN VIVO

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Background. S1P is a lysosphingolipid regulates important biological functions through the interaction with its specific receptors (S1PRs) belonging to the G-protein coupled receptor superfamily; so, it is not surprising that S1P is implicated in a huge range of diseases, including atherosclerosis. In plasma, S1P is associated with the HDL and several studies documented an inverse relationship between HDL levels and the extent of atherosclerotic disease. The HDL atheroprotective effects could be partially attributed to S1P, especially through the stimulation of S1PR1/3, on vascular wall cells. We generated transgenic mice, based on the Cre-LoxP technology, able to overexpress S1PR1/3 in specific tissues to elucidate S1P effects in vivo.
Methods. We obtained S1P1/3 KI mouse models by homologous recombination in ESC, using an optimized targeting vector containing the sequence CAG-Lox-STOP-Lox-S1PR. Transgenes will be only expressed after the removal of Lox-STOP-Lox element by Cre-mediated recombination that will be achieved by crossing S1P1/S1P3 KI mice with ones modified with the Cre recombinase under control of the Lysozyme promoter, a macrophage specific promoter. For the molecular and functional characterization, nucleic acids and proteins were extracted from macrophages and quantified for downstream applications: Real Time PCR, Western Blot and Immunofluorescence to detect receptors, cytokines or cholesterol transporters.
Results. These worldwide unique mouse models permit the tissue-specific overexpression of S1P1/3 receptors causing the amplification of endogenous S1P signaling. Mouse models were fully validated for tissue specific S1PRs overexpression at gene, protein and functional level by performing quantitative Real Time PCR, Western Blot and intracellular cAMP or Ca++ handling assays.
Conclusions. This project selectively targets S1P pathways, gaining insights into the underlying pathophysiological mechanisms. These mouse models may be effectively exploited to investigate the effects of endogenous S1P on the modulation of inflammatory responses associated to the atherogenesis and even in a wide range of chronic inflammatory diseases.

EFFECTS OF MODERATE AND EXCESS ALCOHOL CONSUMPTION ON THE REVERSE CHOLESTEROL TRANSPORT IN VIVO

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Epidemiological studies revealed that moderate and excess alcohol consumption exerts opposite effects on cardiovascular diseases (CVD). Atherosclerotic CVD inversely correlates with the efficiency of RCT, the process resulting in the removal of excess cholesterol from the macrophages of the artery wall.
We aim to evaluate whether moderate and heavy consumption of alcohol may differently impact RCT in an animal model of atherosclerosis-prone mice. RCT was measured with a standardized, radioisotope-based technique in 3 groups of apolipoprotein E knockout mice: group I (n=10), receiving placebo; group II (n=10), receiving 0.8 g/kg/day for 28 days, mimicking a moderate intake of ethanol; group III (n=10), receiving 0.8 g/kg/day for 5 days/week, followed by the administration of 2.8 g/kg/day for 2 days/week, mimicking a heavy intake. Mice were injected with a suspension of 3H-cholesterol loaded macrophages and the radioactivity was quantified in plasma, liver and feces.
Alcohol intake caused a dose-dependent increase in plasma total cholesterol (272 mg/dl+59, 283 mg/dl+53, 374 mg/dl+86 in group I, II and III respectively) and HDL-cholesterol (124 mg/dl+27, 154 mg/dl+37, 179 mg/dl+38 in group I, II and III respectively). Excess alcohol consumption significantly increased plasma LDL cholesterol (126 mg/dl+40, 113 mg/dl+25, 165 mg/dl+65; in group I, II and III respectively) and triglycerides (110 mg/dl+36, 88 mg/dl+16, 136 mg/dl+30; in group I, II and III respectively). The removal of radioactivity from macrophages along the RCT pathway

was higher in animals treated with moderate dose of alcohol: 12.2%+3.1, 15.1%+3.7; 13.3%+2.4; in group I, II and III respectively.
In conclusion, moderate alcohol consumption promotes the mobilization of radioactive cholesterol from macrophages, along the RCT pathway, thus suggesting the amelioration of this process. Differently, excess alcohol consumption seems not to significantly affect the process, but it exerts deleterious effects on plasma lipoprotein profile.

POTENTIAL TRANSLATIONAL ROLE OF LDL APHERESIS PROCEDURES IN AN EXCELLENCE FACILITY: SEARCHING COST-EFFICIENCY AND COST-EFFICACY PARAMETERS, FROM METABOLIC TO INFLAMMATORY PATHWAYS IN ATHEROSCLEROSIS

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The option of therapeutic apheresis in Atherosclorosis has attracted the efforts of several generations of scientists in the last three decades. Starting from the 80s' Federico II University Hospital Excellence Center (EC) for dislypidemias validated an organizational path of care including LDL Apheresis as option for drug resistant FC patients.
In patients with familial hypercholesteremia (FH) most homozygote and some heterozygote who are resistant to drug therapy as well as in other non-FH hypercholesterolemic patients who develop adverse reactions to statin therapy, the desired LDL-C goal cannot be achieved. In these patients, mechanical removal of low-density lipoprotein (LDL) either alone or in adjunction with other hypocholesterolemic drugs may be used. The oldest LDL apheresis method is plasma exchange, wherein LDL is removed by the substitution of whole plasma with albumin. The major drawbacks of this. Procedure are its non selectivity in eliminating not only LDL, but also other important plasma constituents, and complications associated with albumin administration. Thus, it is not recommended as the procedure of choice. The most common selective LDL apheresis methods available today include LDL cholesterol adsorption to dextran sulfate attached to cellulose (DSA) heparin-mediated extracorporeal.
In order to promote as current procedure, LDL apheresis into the core of an EC facility we evaluated efficiency and efficacy determinants, including the actual global path of patients involved.
Consistency of a care setting with technology upgrade is mandatory: given the scale of funding required for clinical development, in a complex path, modest improvements in efficiency could yield substantial savings that may be used also to develop in progress knowledge and research.
Comparison of cost efficiency demonstrated a substantial equivalence of the two procedures with a slight gain in time and tolerance that can balance the slight increase in net costs.
However into the core of an EC, building selective LDL apheresis can reveal some adjunctive opportunities of building cost/efficacy indicators.
First It is now well accepted that atherosclerosis is an inflammatory disease. Besides LDL and HDL (high density lipoproteins),other blood components, such as fibrinogen, C-reactive protein (CRP), homocysteine, leukocytes, and platelets may play an important role in its development. The effect of the various LDL-apheretic methods on these parameters is less known, and comparative data between them remain sparse and deserve further investigations.
Second the service is appropriately allocated and full utilization is attended due to the screening program of the EC that revealed, to date, 5 homozygote and a growing molecular dataset of heterozygote.
Third a translational approach to the inflammatory path can be nowadays be investigated by the Lab facility connected to the Service. Oxidative burst is an innate immune response associated also with marked changes in lipid and lipoprotein metabolism, aimed to neutralize endotoxin toxic effects, on the other hand, recent evidence assigned to NKs a role in proatherogenic processes.
Toward the goal of excellence path of care for FC and drug resistant atherosclerotic patients, LDL aphaeresis inclusion is a significant step; further, add value could be assessed in progress also contributing to state the reduction and the possible relevance of the effects on non-lipid parameters to the clinical outcomes.

MODIFICAZIONI DELL'ASSETTO LIPIDICO IN RAPPORTO AL BODY MASS INDEX IN SOGGETTI NORMOGLUCOTOLLERANTI: DIFFERENZE TRA I GENERI IN POPOLAZIONI NORMOTESE ED IPERTESE

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Introduzione. L'assetto lipidico è notoriamente influenzato non solo da fattori genetici e costituzionali ma soprattutto da aspetti metabolici e dallo stato nutrizionale.
Obiettivo dello studio. In questo studio abbiamo valutato nei due sessi le modificazioni del profilo lipidico al variare del Body Mass Index (BMI) in soggetti normoglucotolleranti, sia normotesi che ipertesi.
Materiali e Metodi. Abbiamo selezionato 945 pazienti (396 M e 549 F) afferenti all'ambulatorio di Ipertensione Arteriosa e Malattie Dismetaboliche della S.C.D.U. Medicina Interna I dell'A.O.U. Maggiore della Carità di Novara. Per evitare le interferenze indotte da un alterato metabolismo glucidico sull'assetto lipidico abbiamo selezionato solo soggetti normoglucotolleranti (NGT) alla OGTT. La popolazione selezionata è stata quindi suddivisa sulla base del BMI in normopeso (BMI <25 kg/m^2), sovrappeso (BMI ≥25 e <30 kg/m^2) e obesi (BMI ≥30 kg/m^2). Una ulteriore suddivisione ha considerato la presenza o meno di ipertensione arteriosa.
Nel sesso maschile (396 soggetti, di cui 284 ipertesi) si sono pertanto selezionati 112 normopeso, 185 sovrappeso e 99 obesi.
Nel sesso femminile (549 soggetti, di cui 359 ipertesi) si sono invece selezionate 242 donne normopeso, 161 donne sovrappeso e 146 donne obese. Di tutti questi pazienti è stata effettuata la valutazione dell'assetto lipidico completo (HDL, LDL, TG, nonCT, apoA1, apoB, apoB/apoA).
Risultati. Nel sesso maschile è stato identificato un peggioramento significativo del profilo lipidico all'aumentare della classe

di BMI, inteso come incremento di TG (p<0.0001), LDL (p<0.05), nonCT (p<0.01) e diminuzione dell'HDL-colesterolo (p<0.01). Considerando i soli ipertesi, questo trend veniva confermato per i TG (p<0.001), il nonCT (p<0.05) e l'HDL (p<0.05). Nel sesso femminile, all'aumentare della classe di BMI si è osservato un incremento di TG (p<0.0001), apoA1 (p<0.05) oltre ad una diminuzione di HDL (p<0.0001); considerando le sole ipertese tale associazione si confermava per i TG (p<0.0001), HDL (p<0.0001), apoA1 (p<0.005), oltre ad un incremento di apoB/apoA1 (p<0.05). Nei soggetti normotesi di entrambi i sessi non abbiamo riscontrato modificazioni significative del profilo lipidico in funzione dell'aumento del BMI.
Conclusioni. Nella nostra popolazione di soggetti NGT abbiamo osservato un progressivo e significativo peggioramento dell'assetto lipidico all'incremento della classe di BMI in entrambi i sessi. Nei maschi risultavano prevalenti le alterazioni a carico di HDL e trigliceridemia, mentre nelle femmine tali alterazioni coinvolgevano anche il metabolismo delle apolipoproteine. Queste associazioni si sono dimostrate valide soltanto nella sottopopolazione di NGT affetti da ipertensione arteriosa.

NEXT GENERATION SEQUENCING APPROACH TO ANALYZE CANDIDATE GENES OF HYPERTRIGLYCERIDEMIA: IDENTIFICATION OF A NOVEL LMF1 MUTATION IN A PATIENT WITH SEVERE HYPERTRIGLYCERIDEMIA

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Objective. Severe hypertriglyceridemia (HTG) is a disorder characterized by plasma triglyceride values >1000 mg/dl (>11.3 mmol/liter). Severe hypertriglyceridemia can be divided into primary and secondary forms. Primary or genetic forms of HTG include mild-to-moderate and severe forms; the rare severe hypertriglyceridemias are thought to be monogenic autosomal recessive and caused by homozygous or compound heterozygous loss of function mutations of few known genes pathophysiologically involved in the intravascular lipolysis of the TG-rich lipoproteins namely lipoprotein lipase (LPL), apolipoprotein CII (APOCII), apolipoprotein AV (APOAV), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1) and GPD1.
Approach and Results. We designed a custom panel for next generation sequencing in order to analyze known genes involved in severe Hypertriglyceridemia by Ion Torrent PGM. Patients with severe HTG were recruited for analysis.
The laboratory workflow consists of the following steps: library construction, preparation of template, run of sequence, data analysis. We used a 314 chip with a range of sequence reads from 400-550 thousand bases. Bioinformatic analysis was conducted by The Ion Reporter System which is a combined hardware and software solution for analyzing human sequencing data.
Conclusions. In this work we describe the workflow and output analysis of a NGS approach for molecular analysis in patients with severe HTG. This sequencing approach led to the discovery of a novel pathogenic mutations of Lipase Maturation Factor 1 (LMF1).

EFFETTO DELLE STATINE SULLA RIGIDITÀ ARTERIOSA: META-ANALISI DEGLI STUDI DI INTERVENTO

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Introduzione. Pochi studi hanno valutato l'effetto delle statine sulla rigidità arteriosa (RA), in aggiunta a quello sui parametri lipidici, ma con risultati non consistenti, spesso a causa del basso numero di partecipanti. Pertanto, scopo di questo lavoro è stato quello di realizzare una meta-analisi degli studi di intervento disponibili, per aumentare il potere di stima dell'effetto delle statine sulla RA.
Materiali e Metodi. Sono stati inclusi gli studi condotti su popolazioni adulte, che riportassero i valori di RA, espressa come velocità dell'onda di polso carotideo-femorale (VOP-cf) basali e finali e che esprimessero i risultati come differenza tra l'utilizzo e non di terapia con statine. Per ogni studio, la differenza tra le medie (DM) e i relativi intervalli di confidenza al 95% (IC95%) sono stati estratti ed inclusi in un modello ad effetto random. Inoltre, sono stati valutati l'eterogeneità ed il bias di pubblicazione ed effettuate le analisi per sottogruppi e la meta-regressione.
Risultati. L'analisi finale ha incluso 9 studi (10 coorti, 436 partecipanti, periodo di intervento compreso tra 8 e 120 settimane). La combinazione dei risultati delle singole coorti ha mostrato che l'utilizzo di statine era associato ad una significativa riduzione di VOP-cf pari a -0.51 m/s (-0.94; -0.10, p=0.02), con una bassa eterogeneità tra gli studi e nessuna evidenza di bias di pubblicazione. L'analisi per sottogruppi e la meta-regressione non hanno individuato significative fonti di eterogeneità.
In conclusione, questa meta-analisi suggerisce che l'utilizzo di statine riduce la RA. Questo effetto favorevole appare almeno in parte indipendente dalle concomitanti variazioni del profilo lipidico e dalla durata dell'intervento.

BYGLICAN EXPRESSION, ARTERIAL STIFFNESS AND PROATHEROGENIC PROFILE IN FORMER SMOKERS AFTER 1-YEAR SMOKE CESSATION

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Background. Young cigarette smokers may already present early signs of vascular inflammation and damage; Biglycan (BGN) has been shown to play a critical role in the initiation and progression of vascular lesions, also in young smokers. We investigate whether after smoke cessation monocyte BGN expression is reduced; moreover, we evaluated any improvement of pro-atherogenic profile and of arterial stiffness (AS), and their relationships with BGN.
Methods. We had been already enrolled 251 young people who had decided to quit smoking; of these, 71 have completed the 12 months observation period. At enrollment and 12 months later we evaluated anthropometrics, laboratory profile, pulse wave velocity (PWV), augmentation index (AIx), carotid intima-media thickness (cIMT), BGN expression.

Results. After 12 months smoke abstinence we found a significant decrease of inflammatory markers (Hs-CRP: -23.3%; Fibrinogen: -11.8%; IL-6: -9.2%), and increased HDL-C levels (+9.3%); blood pressure values were also slightly reduced. PWV (-6.55%) and AIx (-20.1%) appeared to be improved; cIMT remained unchanged. BGN expression appeared to be reduced (-42.8% relative reduction). BGN reduction appeared to be correlated with fibrinogen and IL-6 reduction, and also with PWV reduction. However, reduced PWV and AIx appeared to be not dependent on Δ BGN by the multiple regression analysis.
Conclusion. After 1 year of abstinence the levels of IL-6, CRP, Fibrinogen, HDL-C, and BGN expression, as well AS indices, are significantly improved as compared to baseline. This is the first evidence that removing exposure to a well-known cardiovascular risk factor, such as cigarette smoking, even the expression of monocyte BGN is significantly reduced.

ATEROGENESI, DEPRESSIONE PSICHICA E LESIONI PRENEOPLASTICHE COLORETTALI: EVIDENZE DI UNA CORRELAZIONE SIGNIFICATIVA

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Introduzione. Recenti evidenze scientifiche mostrano importanti connessioni biomolecolari tra asse intestino-cervello (gut-brain axis) e aterogenesi, tramite la modulazione dalla risposta infiammatoria sistemica e locale (1). La presenza di soprappeso/obesità si correla inoltre positivamente ad un aumentato rischio di carcinoma colorettale così come a comorbidità psichiatriche (2-4). Sulla linea di questa innovativa visione integrata metabolica-psichica-intestinale, abbiamo delineato uno studio clinico trasversale mirato ad evidenziare eventuali connessioni tra grado di aterogenesi precoce, tratti ansioso-depressivi e presenza di lesioni adenomatose coliche.
Materiali e Metodi. Studio clinico trasversale caso-controllo con arruolamento di pazienti in attesa di colonscopia per sangue occulto fecale positivo con anamnesi negativa per neoplasie e/o malattie infiammatorie intestinali. Coloro che aderiscono allo studio vengono sottoposti a rilevazione dei parametri antropometrici e metabolici (altezza, peso, circonferenza vita e fianchi, pressione arteriosa, glicemia a digiuno), ad ecocolordoppler dei tronchisovraortici per il calcolo dello spessore medio-intimale (IMT), ed a test psicometrici per evidenziare ansia o depressione (HADS). In base all'esito della colonscopia i soggetti vanno a costituire due gruppi (presenza o assenza di polipi adenomatosi). Il presente studio, approvato dal Comitato Etico locale, è iniziato a gennaio 2015. Analisi statistica con test appropriati usando software SigmaPlot v.12.
Risultati. 18 pazienti arruolati (M/F 8/10, età compresa tra 48-77 anni, media 62.6). 10 pazienti (M/F 6/4) hanno riscontro di almeno un polipo adenomatoso alla colonscopia. I seguenti test sono risultati statisticamente differenti tra i due gruppi (presenza/assenza di adenomi colorettali):
- circonferenza vita (adenoma vs no-adenoma: media ±DS 105.2 cm ±13.4 vs 89.5 cm ±4.7, p=0.009);
- Q-IMT carotideo (adenoma vs no-adenoma, mediana 793 vs 638 micrometri; percentile 25%: 724 vs 588 micrometri; percentile 75%: 1106 vs 769; p=0.037);
- peso corporeo (adenoma vs no-adenoma: 66.4 kg ±8.7 vs 80.9 kg ±15.3, p=0.03);
- punteggio HADS per depressione (adenoma vs no-adenoma punteggio medio±DS 4.9±3,2 vs 1.7±1,8, p=0.026).

Conclusioni. I risultati iniziali stanno mostrando un interessante quadro di correlazione tra aterogenesi precoce, presenza di tratti psichici depressivi e lesioni preneoplastiche colorettali.
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MICROPARTICELLE DI DERIVAZIONE IMMUNITARIA ED ATEROSCLEROSI PRE-CLINICA NEL PAZIENTE DISLIPIDEMICO E NEL GRANDE OBESO

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Introduzione. I pazienti dismetabolici presentano una precoce alterazione degli indicatori pre-clinici di danno vascolare. Si ritiene che i linfociti iNKT ed i monociti CD14+/CD16+ possano esercitare un ruolo rilevante nella promozione del processo aterosclerotico. Il significato delle microparticelle (MP) derivate da tali popolazioni cellulari non è noto. Lo scopo del nostro lavoro è stato quello di valutare, in pazienti dislipidemici ed obesi, la relazione tra il numero di MP plasmatiche derivate da linfociti iNKT e monociti CD14+/CD16+ e lo spessore medio-intimale (IMT) carotideo, indicatore pre-clinico di danno vascolare.
Materiali e Metodi. Sono stati reclutati 25 pazienti dislipidemici, 20 pazienti con grave obesità (BMI medio: 48,3 kg/m^2) e 10 controlli sani. In tutti i partecipanti è stata effettuata la misurazione della pressione arteriosa, dell'altezza, del peso, della circonferenza vita e la determinazione di glicemia, colesterolo totale, LDL, HDL, trigliceridi, GOT, GPT, GGT. Il grado di steatosi epatica e l'area del grasso viscerale sono stati determinati mediante ecografia. L'IMT è stato misurato mediante ultrasonografia B-mode ad alta risoluzione a livello della carotide comune. Il numero delle MP CD3+/iNKT e CD14+/CD16+ è stato calcolato mediante analisi citofluorimetrica.
Risultati. Rispetto ai controlli, le MP CD3+/iNKT risultano significativamente aumentate sia nei pazienti dislipidemici ($p<0,01$) che negli obesi ($p<0,01$); un aumento significativo si è riscontrato anche per le MP CD14+/CD16+ tanto nei pazienti dislipidemici ($p<0,05$) quanto negli obesi ($p<0,01$). Il numero di MP CD3+/iNKT risulta essere correlato positivamente con il BMI ($p<0,05$) e con l'IMT ($p<0,05$); il numero di MP CD14+/CD16+ correla positivamente con il BMI ($p<0,05$), con l'area del grasso viscerale ($p<0,05$), con il grado di steatosi epatica ($p<0,05$) e con l'IMT ($p=0,05$). La correlazione tra MP e IMT si rafforza dopo correzione per età e sesso ($p<0,01$ sia per le MP CD3+/iNKT che per le MP CD14+/CD16+). All'analisi multivariata il numero di MP CD3+/iNKT ($p<0,05$) e il numero di MP CD14+/CD16+ ($p<0,05$) risultano essere predittori

indipendenti dell'IMT (altre variabili incluse nel modello erano: età, sesso, fumo, pressione arteriosa sistolica, colesterolo totale, diabete).
Discussione. Nel nostro studio, il numero di MP plasmatiche derivate da due distinti gruppi leucocitari, linfociti iNKT e monociti CD14+/CD16+, risulta essere predittore indipendente dell'IMT carotideo. In pazienti dislipidemici ed obesi, il riscontro di livelli più elevati di MP derivate da iNKT e monociti potrebbe riflettere il livello di attivazione di tali popolazioni cellulari nello stato di infiammazione cronica di basso grado tipico delle condizioni dismetaboliche. È noto che l'ispessimento dell'IMT rappresenta un importante predittore indipendente di mortalità cardiovascolare, pertanto la determinazione dei livelli plasmatici di MP di derivazione immunitaria nei pazienti dislipidemici e nei grandi obesi potrebbe fornire utili informazioni aggiuntive per una più accurata stratificazione del rischio cardiovascolare.

VALIDATION OF THE PLAC TEST FOR THE MEASUREMENT OF LP-PLA2 ACTIVITY ON THE DIMENSION VISTA ANALYZER

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Introduction. Lipoprotein-associated phospholipase A2 (Lp-PLA_2) is synthesized by macrophages and other inflammatory cells and circulates in human blood primarily bound to low-density lipoproteins, with a smaller amount associated with high-density lipoproteins. Lp-PLA_2 is a specific marker of vascular inflammation and is associated with incident and prevalent coronary heart disease and incident stroke, independently of traditional cardiovascular risk factors. Measurement of Lp-PLA_2 activity is recommended for patients at high risk for cardiovascular disease and the PLAC® Test Activity kit is available for routine laboratory practice. Here we describe the validation of the PLAC® Test on the Siemens Dimension Vista® 1500 analyzer.
Methods. Precision, accuracy, linearity, sensitivity, interference, substrate depletion, and inhibitor effect were assessed on 40 serum samples supplied by diaDexus. Lp-PLA_2 activity was also measured in 250 apparently healthy donors (123 males, 127 females; mean age 38 years, range 18-70) and correlation between Lp-PLA_2 activity and other blood markers was evaluated.
Results. A strong linear relationship was found between measurements performed on the Dimension Vista® and the corresponding values reported in the PLAC® Test kit by the supplier, which were obtained using a Beckman Coulter AU400® analyzer. Reference intervals (mean +/- 2SD) for serum Lp-PLA_2 activity were 123.1–282.3 nmol/min/ml for males and 87.3-235.3 nmol/min/ml for females. Intra-assay and inter-assay precision ranged from 0.6% to 1.4% and from 0.9% to 3.0%, respectively. Accuracy showed an R^2=0.999. Linearity replicates all showed R^2>0.98. Sensitivity was <10 nmol/min/ml. No interference was observed for hemoglobin up to 1.5 mg/ml, bilirubin up to 20 mg/dl, albumin up to 90 g/L, and Intralipid® up to 2.5 mg/ml. A highly significant correlation (r=0.29, P<0.0001) was found between Lp-PLA_2 activity and total cholesterol levels.
Conclusions. Validation of the PLAC® test on Siemens Dimension Vista®, a high throughput system, might allow large scale epidemiological investigations.

PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 KNOCK OUT MICE ARE PROTECTED FROM NEOTINTIMAL FORMATION IN RESPONSE TO PERIVASCULAR CAROTID COLLAR PLACEMENT

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We have previously shown that Proprotein convertase subtilisin kexin type 9 (PCSK9) is expressed in cultured smooth muscle cells (SMCs) and it is detectable in human carotid atherosclerotic plaques. In the present study we compare the vascular changes in response to perivascular carotid collar placement of PCSK9 knock-out (PCSK9-/-) and wild type littermate (PCSK9+/+) mice. PCSK9-/- mice (n=15 per group) showed a less marked intimal thickening compared to WT mice, a decreased intimal media ratio and tendency to higher lumen area. Carotid lesions of WT mice had an elevated content of SMCs and collagen, while no difference in macrophage content was detected between the two groups. Cultured SMCs PCSK9-/- exhibited a predominant contractile phenotype compared to SMCs PCSK9+/+ (with increased mRNA levels of contractile marker such as Smooth Muscle α-actin and reduced levels of synthetic marker such as caldesmon and Col 1a1). The absence of PCSK9 reduced the proliferation rate (determined by cell counting and iCelligene real time measuring), and impaired migratory response to platelet derived growth factor-BB (PDGF-BB). The reconstitution of PCSK9 expression in SMCs PCSK9-/- restored the synthetic phenotype (downregulation of α-SMA and induction of caldesmon) and increased proliferation rate compared to SMCs PCSK9-/- (doubling time 41.2±1.9 h vs 32.2±3.1 h). Finally, in response to PDGF-BB, the morphological changes of PCSK9-/- mSMCs (measured by iCelligence monitoring and cytoskeletal staining) was significantly impaired compared to PCSK9+/+ cells. Taken together, the present results suggest a favorable action of PCSK9 on neointima formation in response to perivascular carotid.

ANGPTL3 DEFICIENCY MARKEDLY ATTENUATES POSTPRANDIAL LIPID RESPONSE: IMPLICATIONS FOR THE MECHANISMS OF FAMILIAR COMBINED HYPOLIPIDEMIA (FHBL2)

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Introduction. Homozygosity of loss-of-function mutation (LOF) in human ANGPTL3 gene (p.S17*) is a direct cause of familial combined hypolipidemia (FHBL2, OMIM #605019) featuring low plasma VLDL, LDL and HDL levels. Angptl3 deficiency is linked to activation of lipoprotein lipase and improved insulin sensitivity. It

was therefore of interest to characterize the postprandial response to a high fat meal in p.S17* LOF-carriers.
Methods. We obtained plasma samples from 7 homozygous carriers (undetectable circulating Angptl3) (Angptl3-/-), 31 heterozygote carriers (50% circulating Angptl3) (Angptl3-/+) and 35 non-carriers (controls) during fasting and 2, 4 and 6 hrs after a high fat meal. Plasma samples were measured for total lipids, TG-rich lipoproteins (TRL, d<10.19 g/ml), NEFA, Angptl3 and 4, chylomicron apoB-48, apoB-100, apoA-I and HDL, glucose and insulin. Postprandial changes were estimated by incremental area under the curves (iAUC) and comparisons between groups were performed after adjustment for age, sex and body mass index.
Results. At baseline, Angptl3-/- subjects were older and showed slightly increased fat mass compared to controls. As expected, they presented significantly lower levels of plasma TG, LDL-C, HDL-C, apoB-100 and apoA-I. Compared to controls, Angptl3-/-subjects showed markedly lower postprandial iAUC of total TG (-85%, p=0.019), TRL (-83%, p=0.019) and apoB-48 (-71.3%; p=0.285). Also Angptl3-/+ subjects displayed a tendency to a lower postprandial lipid response even though the differences did not reach statistical significance. However, in these subjects an early decrease of TG and TRL levels that started to fall already after 2 hours was observed. No significant differences in postprandial iAUC of NEFA, glucose and insulin were detected. Interestingly, in Angptl3 -/+ and control subjects, plasma Angptl3 showed a steady decrease during postprandial phase reaching 28% (p<0.001) lower levels at 6 hrs compared to baseline; on the contrary, no significant postprandial changes in plasma levels of Angptl4 were observed. In the whole group of study subjects, baseline plasma levels of Angptl3 showed a significant correlation with iAUC of TG (r=0.289, p=0.013), TRL (r=0.239, p=0.042) and apoB-48 (r=0.236, p=0.047).
Conclusions. The present data clearly demonstrated that in subjects with Angptl3 deficiency postprandial lipid levels remain low after high fat diet and showed a faster turnover rate compared to controls, indicating that changes in postprandial lipid metabolism might be one important part of mechanisms underlying FHBL2. Moreover, our findings indicate that the total Angptl3 deficiency markedly improves postprandial lipemia and this might be associated with a potentially anti-atherogenic phenotype.

DETECTION OF FAMILIAL HYPERCHOLESTEROLEMIA IN A UNSELECTED COHORT OF ITALIAN HYPERCHOLESTEROLEMIC CHILDREN: RESULTS OF A FAMILY AND DNA BASED SCREENING

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Background. Familial Hypercholesterolemia (FH) is the most common dyslipidemia in childhood. Early detection and treatment of FH has been advocated to effectively prevent the development of atherosclerosis in affected individuals. However, the diagnosis of FH in children remain difficult, so that a child with FH is mainly detected through parents with already diagnosed FH, a method referred as cascade screening. Recently, EAS proposed FH diagnostic criteria for children based on LDL-C cut-off values and family history of hypercholesterolemia and/or premature cardiovascular disease. However, their effectiveness in identifying FH in unselected hypercholesterolemic children have not been carefully determined.
Methods and Results. Here, we report the results of a screening program for FH in a cohort of 227 families collected throughout a hypercholesterolemic (HC) proband (LDL-C>95 th age and sex specific percentile). In HC children (age range 2-15 years; mean 8.3±3.4 years) the clinical suspicion of FH was based upon the present of at least one parent with LDL-C>95th percentile and TG<75° and/or premature cardiovascular disease (CVD). The diagnosis of FH was definitively confirmed by resequencing of LDLR gene. Based upon the selected clinical criteria, 78 children (34.4%) were classified as suspected FH. Among them, 38 (47.4%) were found to carrying FH-causing mutations in the LDLR gene. A total of 27 different LDLR mutations were identified and all were present at heterozygous state. Of them, 17 were missense, 1 was nonsense, 4 were frameshift, 2 were in-frame deletions and 2 were splite site variations. Two mutations were novel (p.Leu547Pro and c.940+2delGAGT) while the others were already reported in the literature. When plasma lipids were compared between children with and without LDLR mutations, the former showed significantly higher plasma levels of LDL-C (215.2±52.7 mg/dl vs 181.0±44.6 mg/dl, P<0.001) and apoB (131.6±38.3 mg/dl vs 100.3±30.0 mg/dl, P<0.004) and lower of HDL-C (48.4±13.7 vs 55.5±13.1, P=0.042). It is interesting to note that children without LDLR mutations were slightly overweight (P=0.04) and showed higher plasma insulin levels (P=0.026), as compared to those carrying FH-causing alleles. When children with suspected FH were distributed within the different categories of EAS criteria:
1) LDL-C >160 mg/dl;
2) LDL-C >160 mg/dl and family history of CVD ± baseline high cholesterol in one parent;
3) LDL-C value >190 mg/dl, the last one provided to be the most reliable for detecting FH. In fact, it allowed identifying 69% of definite carriers of FH-causing alleles.

Conclusions. These findings indicate that in unselected hypercholesterolemic children LDL-C levels above 190 mg/dl associated with a positive family history of hypercholesterolemia appeared to be most reliable criteria for detecting FH. However, as about 30% of suspected FH children do not carry FH-causing allele, our results also underlined the importance of genetic testing in children with clinical suspicion of FH.

REGIONAL DIFFERENCES IN GENE EXPRESSION BY HUMAN SUBCUTANEOUS ADIPOSE TISSUE

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Introduction. Obesity, defined as abnormal or excessive fat accumulation, increases risk for multiple metabolic diseases, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Subcutaneous adipose tissues (SAT) store represent over 80% of total body fat, and the most studied subcutaneous depots are the abdominal and gluteofemoral, while visceral adipose tissue (VAT) represent 5-20%. Regional differences in cellular composition, microvasculature, metabolic characteristics, and adipokine production have been documented. The initial reports of J. Vague

over 60 years ago made clear that in addition to total fat mass, the pattern of fat deposition is independently linked to disease risk. Central obesity, particularly visceral obesity, but also including fat accumulation in abdominal subcutaneous (apple-shaped, android), confers increased risk for metabolic complications of obesity and even all-cause mortality; whereas lower or peripheral obesity (pear-shaped, gynoid), is associated with lower risk and may be protective. Depot differences in adipocyte metabolism and endocrine function are clearly important in etiology of obesity-related diseases, and the relative contribution of VAT compared to abdominal SAT is still controversial. To a lesser extent, the heterogeneity between the different subcutaneous depots has also been investigated: the gluteofemoral adipose tissue depot displays differential fatty acid handling compared to the subcutaneous abdominal depot. Several clinical and experimental studies indicate that different fat depots arise from distinct precursors, derived from mesenchymal stem cells, with inherently different proliferative and adipogenic properties. New data from Karastergiou et al. suggest that the profound functional differences between the upper-body and lower-body tissues are controlled by site-specific sets of developmental genes, such as homebox (HOX) genes, that control the body plan of an embryo along the anterior-posterior axis, and are under epigenetic control. Here, we studied global mRNA expression in gluteal and abdominal subcutaneous adipose tissues in eight human subjects from the BEDREST study, with the objective of characterising the global mRNA molecular phenotypic profile in these two adipose depots.

Methods. Eight healthy middle aged men [age 60±3 yr, (57-65)] underwent subcutaneous tissue biopsy from abdomen and gluteus. RNA was isolated, than labeling and hybridization on microRNA microarray chips were performed. Statistical significance of expression differences was calculated using a one-factor ANOVA test. To assess similarities and differences among gene expression profiles, hierarchical clustering was performed.

Results. In the microarray analysis, 42.405 probes were detected in abdominal and gluteal adipose tissues. Considering 1.5 fold change as lower limit, a total of 181 probes were differentially expressed between the abdominal and gluteal depot, corresponding for a total of 142 coding genes. Most of the expression differences were modest, 85% in 1.5-3 fold change range. Gene ontology and pathway analysis identified HOX genes expression differences between gluteal and abdominal depots, with HOXA13, HOXA11, HOXC10, HOXC12 down-regulated, and IRX2, HOXA3, HOXB7, HOXA5, HOB8, HOXD3, HOXA6 up-regulated in abdominal versus gluteal adipose tissue.

Conclusions. Body fat distribution is an important metabolic and cardiovascular risk factor, because the proportion of abdominal to gluteofemoral body fat correlates with obesity-associated diseases and mortality. There is evidence that different adipose tissue depots have different morphology, physiology and adipokine profiles. The current data are consistent with previous work pointing to the importance of developmental genes, such as HOX genes, in the depot-specific functional and morphological differences of human adipose tissues. In this study we find new evidence that subcutaneous abdominal and gluteal adipose tissue show differences in gene expression, with particular focus on HOX genes. Our results confirm that epigenetic regulation of HOX genes contributes to depot-specific functional characteristics of SAT.

PENTRAXIN 3 PLAYS A KEY ROLE IN THE IMMUNOMODULATION OF DIET INDUCED-OBESITY IN MICE

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Aim. Metabolic syndrome and obesity are characterized by chronic low grade inflammation. Aim of our work was to study the role of PTX3, an essential component of the humoral arm of innate immunity, during high fat diet induced obesity.

Methods. PTX3 KO and WT littermates were fed a HFD (45% Kcal from fat) for 20 weeks. Body weight was measured weekly; fat distribution (magnetic resonance for imaging, MRI) and glycaemia (glucose-GTT and insulin-ITT tolerance tests) was checked at 10 and 20 weeks. Immunophenotyping of adipose tissue was performed at 20 weeks.

Results. Compared to WT, PTX3 KO mice showed a decreased weight gain during HFD diet (AUC weight gain 182.91 vs 147.22; $p<0.05$), coupled to a decreased accumulation of fat at both 10 and 20 weeks in the visceral(VAT) and subcutaneous adipose deposits(SCAT) ($p<0.05$) measured by MRI. Furthermore, VAT and SCAT percentage on body weight was significantly lower (7,04% vs 4,66% and 5,75% vs 3,61% respectively; $p<0.05$) compared to liver, pancreas and spleen and associated to a decreased adipocyte areas in PTX3 KO VAT ($p<0.01$). Basal glycaemia at both 10 and 20 weeks was similar between groups as well as glucose and insulin tolerance measured by GTT and ITT, excluding a direct role of PTX3 on glucose homeostasis. As PTX3 is a key component of innate immunity, we focused our attention on the inflammatory response in VAT of PTX3 KO: qPCR showed a decreased expression of MCP-1 and IL-6 and increased VEGF and CD31 associated to a reduced infiltration of monocytes and macrophages ($p<0.05$). Macrophages sorted from VAT showed enhanced expression of molecules associated with M2-like polarization (Arg, YM-1).

Conclusion. Deficiency of PTX3 results in reduced HFD induced obesity. This effect appears to be the consequence of increased polarization of macrophages toward an M2 phenotype.

DYNAMICS CHANGES IN ENDOTHELIAL EXTRACELLULAR MATRIX INDUCED BY VERY LOW DENSITY LIPOPROTEIN

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Previously we described the vascular extracellular matrix remodeling induced by normal VLDL at physiological levels

1) Particularly, we described the differences in chondroitin sulfate/dermatan sulfate proteoglycans (CS/DS-PGs) according to the endothelial cell phenotypes.

2) The aim of the present study was to analyze the expression pattern of CS/DS-PGs in the presence of increasing levels of N-VLDL. Human N-VLDL were isolated by ultracentrifugation from healthy volunteers. Human umbilical vein endothelial cells (HUVEC) were obtained and cultured as described by Ulrich-Merzenic.
3) Then, HUVEC were incubated with 0,75 and 100 mcg/mL of lipoprotein for 24 h.

Protocols were approved by the Bioethics Committee of the University of Buenos Aires, Argentina. After treatment, CS/DS-PGs were characterized through:

1) PG core protein secretion, specifically decorin, biglycan, and versican analysis by immunoblot;
2) glycosaminoglycans (GAGs) content studied by reverse phase HPLC;
3) the levels of chondroitin N-acetylgalactosaminyltransferase-2 (ChGn-2) and chondroitin-4-O-sulfotransferase-1(C4ST-1) mRNA by RT-PCR.

A significant increase in the protein core of decorin and biglycan was detected after treatment (0 vs 75 and 0 vs 100 mcg/mL N-VLDL, $p<0.05$, n=3), whereas for versican the increase was only observed at 75 mcg/mL (0 vs 75 mcg/mL N-VLDL, $p<0.001$, n=3). A significant increase in CS and DS chains was detected at both levels of N-VLDL (38.5±15.0; 388.0±20.0 and 82.5±50.0 ng/mL; 0, 75 and 100 mcg/mL N-VLDL, $p<0.05$, n=3), accompanied by an increase in the sulfation ratio 4S/0S of CS and DS chains (4.88±0.13; 13.97±1.8; 14.53±11.46; 0, 75 y 100 mcg/mL, n=3). No differences were observed in ChGn-2 and C4ST-1. At physiological levels, VLDL induced a CS/DS-PG secretion pattern that may contribute to the atheroprotective properties of this endothelial phenotype; such characteristics were lost in the presence of higher levels of the lipoprotein. Our results highlight the importance of CS/DS PGs as a new target for atherosclerosis treatment.

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DEPLETION IN LPA-I:A-II PARTICLES ENHANCES ENDOTHELIAL PROTECTION BY HDL IN GENETIC LCAT DEFICIENCY

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LCAT deficiency is a rare disorder characterized by reduced plasma cholesterol esterification, LCAT activity, HDL cholesterol and apoA-I levels, and enhanced unesterified/total cholesterol ratio. The severe deficiency of atheroprotective HDL in carriers of LCAT deficiency should increase their risk of developing coronary heart disease (CHD); however, subjects with LCAT deficiency do not show remarkably increased preclinical atherosclerosis. The various HDL subpopulations are differently efficient in maintaining endothelial cell homeostasis and genetic HDL disorders represent a unique tool to understand the relationship between HDL quantity and quality and HDL function. In the present study, we evaluated the vasoprotective effects of HDL isolated from carriers of LCAT deficiency, which are characterized by a selective depletion of large LpA-I:A-II particles and predominance of small, pre- β migrating HDL. For this purpose HDL from LCAT-deficient carriers and controls were isolated by ultracentrifugation and characterized on the basis of their size, shape, surface charge and apolipoprotein composition. Anti-inflammatory and vasoprotective properties of HDL were tested in endothelial cells. In addition, plasma adhesion molecule levels and flow-mediated vasodilation were measured in subjects with LCAT deficiency and controls. In endothelial cells, HDL from LCAT deficient carriers showed increased anti-inflammatory properties and enhanced capacity to promote eNOS activation and consequently NO production with a gene dose-related effect. In agreement with the in vitro data, carriers of LCAT mutations have flow-mediated vasodilation values comparable to control subjects despite the low plasma HDL levels. The enhanced vasoactive action of carrier HDL could be explained by the selective depletion in LpA-I:A-II particles, which are less effective than LpAI particles in modulating NO production. The in vitro and in vivo data described in the present work suggest that the specific HDL subpopulations which accumulate in LCAT deficiency are more effective in maintaining endothelial cell homeostasis.

THE ROLE OF LP(A) IN RESTENOSIS AFTER PRIMARY CORONARY PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY: A CASE REPORT

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Angiographically documented restenosis after primary coronary percutaneous transluminal angioplasty (follow-up diameter stenosis >50%) still occurs in 20% to 30% of patients and clinical restenosis (recurrent angina due to restenosis in the treated segment) developed in 10% to 15% of patients in the first year after treatment. The pathogenesis of restenosis in response to mechanical injury is not completely understood. The development of neointimal thickening as a result of migration and stimulation of smooth muscle by growth factors and the elastic properties of the vessel undergoing PTCA and its recoil in the development of restenosis have received attention. Clinical variables that appear to be associated with increased rate of restenosis include diabetes, severe angina, male sex, smoking, and older age.

Several studies investigated the influence of thrombotic risk factors, such as hyperhomocysteinemia, hyperfibrinogenemia, presence of factor V Leiden, LAC, prothrombin G20210A heterozygosity, MTFHR C677T heterozygosity, ACE I/D and AT1R A1166C polymorphisms on restenosis after PTCA.

We describe a case of 37 years old Caucasian man who presented unstable angina on October 2012, he was treated with a percutaneous transluminal coronary angioplasty and six months after he had a restenosis. He had a cluster of risk factors such as overweight,

smoking, premature family history for myocardial infarction. In April 2013 despite of both lipid lowering diet and treatment, antiplatelet therapy and smoking cessation a second attack of unstable angina occurred. No thrombotic risk factors were detected; LDL cholesterol level was 26 mg/dl and the only significant risk factor detected was an high level of Lp(a) (1470 mg/dl).
In conclusion Lp(a) determination could be an important tool in secondary and even primary prevention; particularly according to 2010 EAS guidelines, we suggest to determine serum Lp(a) levels in patient with premature cardiovascular disease, familial hypercholesterolemia, and cardiovascular risk event >3%.

ASSOCIATION BETWEEN LP(A) AND ATHEROSCLEROSIS IN MENOPAUSAL WOMEN WITHOUT METABOLIC SYNDROME

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The association between Lp(a) and Common Carotid Intima Media Thickness (IMT), a marker of early atherosclerosis, has been evaluated in 222 menopausal women living in the area of Naples (Italy) participating to a large (N=5.062) epidemiological Study from 1993 (Progetto Atena).
Lp(a) was significantly lower in women with Metabolic Syndrome (MS) (19.1 mg/dL vs. 27.9 mg/dL, p<0.05). Lp(a) was negatively correlated with Waist Circumference (WC) (p=0.023), Insulin (p<0.001), and Homa (p<0.001), and positively with LDL (p<0.022). In univariate logistic analysis, Lp (a) was associated with increased IMT (≥1.30) (OR 1.02; p=0.005). In multivariate analysis Lp(a) showed the following OR of having common carotid IMT (≥1.30 mm): 1.03 (p=0.003), adjusted for age, LDL, WC; 1.02 (p=0.019), adjusted for age LDL, HOMA. In women without MS (N=128), after controlling for Age, LDL and WC, we found the following OR for increased IMT (≥1.30) [OR 1.03; p=0.006 for Lp(a)] and after controlling for age, LDL and Homa OR [1.02; p=0.021 for Lp(a)]. In women with MS (N=94) these relationships were not statistically significant. Quantitative measurement of Lp(a) gives useful information in the risk assessment for atherosclerotic disease, in particular in menopausal women without MS, by identifying a subgroup with high Lp (a) and early atherosclerosis, at higher risk for cardiovascular events.

DIETARY INTERVENTION TRIAL FOR CARDIOVASCULAR PREVENTION: MEDITERRANEAN DIET VS. VEGETARIAN DIET

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Background. Nutrition is able to alter the cardiovascular health of the general population. Actually, the optimal dietary strategy for cardiovascular prevention is yet far to be defined. Mediterranean and vegetarian diets are those reporting the greatest grade of evidence in the literature, but no experimental studies comparing these two dietary patterns are available.
Aim. To conduct a dietary intervention trial with the use of two different diets, Vegetarian and Mediterranean, in order to compare the effects of these diets on several anthropometric and circulating cardiovascular biomarkers.
Methods. Forty-six clinically healthy subjects (33 F; 10 M; mean age: 50.6±12.1) were randomly allocated to Mediterranean (Med) or Vegetarian (Veg) isocaloric diets lasting three months each, and then crossed over. Adherence to the specific dietary intervention was established through questionnaires and 24 h dietary recall. Anthropometric measurements, body composition and blood sampling were obtained from each participant at the beginning and at the end of each intervention phase.
Results. Subjects were enrolled to start with either Veg (n=23) or Med (n=20). Med and Veg both determined a significant (p<0.05) reduction of total body weight and fat mass at 3 months, without any difference between the two types of diets [body weight: -3.6 kg (-4.6%) for Med and -3.5 kg (-4.3%) for Veg], [fat mass: -3.0 kg (-11.4%) for Med and -2.6 kg (-7.0%) for Veg]. With regard to biomarkers, a significant (p<0.05) decrease in total cholesterol levels and glucose was observed in both groups [total cholesterol: -10.7 mg/dL (-5.0%) for Med and -18.7 mg/dL (-8.8%) for Veg], [glucose: -0.04 g/L (-4.4%) for Med and -0.01 g/L (-1.2%) for Veg], whereas a significant reduction in LDL cholesterol levels was observed only in Veg [-13.1 mg/dL (-10.0%)] and not in Med. Furthermore, insulin levels significantly decreased only in Med [-2.8 mU/L (-24.1%); p<0.05].
Conclusions. Mediterranean and Vegetarian dietary patterns appears to be equally effective in reducing anthropometric and circulating risk factors for cardiovascular disease among clinically healthy subject.

METABOLIC DISTURBANCES AND RISK FOR CANCER IN THE 25 YEARS FOLLOW-UP OF THE VENTIMIGLIA HEART STUDY. NO EFFECT OF THE RS1137101 (LEPR) AND RS1501299 (ADQ) POLIMORPHISMS

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Obesity and Metabolic syndrome (MetS) have been associated to the risk of developing Cancer. The cancerogenic effects of MetS seems to be mediated by inflammation but also by the actions of adiponectin (ADQ) and leptin (LEP), whose levels are altered in different metabolic disturbances. ADQ shows anti-inflammatory and anti-atherogenic effects while LEP acts regulating energy balance but is also involved in immune-and inflammatory responses. Different polymorphisms of ADQ and LEP and their receptors (ADQR and LEPR) have been associated to Cancer.
In this study we analyzed data regarding the incidence of cancer in the 25 years follow-up of the Ventimiglia Heart Study (VHS) epidemiological project. We selected all subjects in the 25-75 years range of age. We evaluated different metabolic parameters at baseline as predictors of Cancer in the follow up years. To reduce the num-

bers of confounding and spurious variables we first detected those parameters that were associated with different plasma levels (or risk factors that showed different prevalence) between subjects that developed Cancer and those who survived Cancer. We then detected best discriminant threshold by ROC analysis and finally we evaluated univariate and multivariate hazards ratios (HR) and confidence intervals (CI) for Cancer risk. Finally we evaluated the effects of the ADQ rs1501299 and LEPTR rs1137101 SNPs effects on Cancer survival.
At the end of the selection, the best predictor were Age (yrs classes) with HR(CI) =2.2(1.2-4.1) for 45-65 yrs, 3.4(1.8-6.5) for 65-75 yrs, male gender HR=2.3(1.5-3.6), TG/HDL ratio (log) HR=2.3(1.5-3.6), Waist Circumference MetS criteria (WC) HR =1.6(1.0-2.5), FBG >93 mg/dL HR =1.5(1.0-2.3). At multivariate analysis WC failed as independent predictor. Both investigated SNPs were not affecting cancer survival.
In conclusion metabolic disturbances predicted cancer in a 25 years follow up of a Sicilian population, and adipokines polymorphisms were not able to explain the results.

DIAGNOSI CLINICA E MOLECOLARE DI FH: RISULTATI PRELIMINARI DELLA CASISTICA RACCOLTA NELL'AMBULATORIO DISLIPIDEMIE DEL POLICLINICO SANT'ORSOLA-MALPIGHI DI BOLOGNA

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Da alcuni anni il nostro Centro, nell'ambito del progetto LIPIGEN, sta iniziando a sottoporre ad indagine genetica i soggetti verosimilmente affetti da ipercolesterolemia familiare, con particolare attenzione a quelli in età pediatrica, per i quali è stato peraltro messo a punto quest'anno un PDTA multidisciplinare dedicato.
Scopo dello studio è stata un'analisi preliminare dei dati finora raccolti riguardo i pazienti sottoposti a ricerca di mutazioni nel gene per il recettore delle LDL.
Sono stati arruolati 88 soggetti (46 femmine e 42 maschi) appartenenti a 23 famiglie, di cui 27 sotto ai 18 anni e sottoposti, previa acquisizione del consenso informato, a prelievo di sangue per il dosaggio di: colesterolemia totale, trigliceridi, HDL, LDL, ApoA1, ApoB, creatinina, CPK, glicemia, uricemia, transaminasi. Sulla base dei dati clinici e anamnestici, ai soggetti è stato attribuito un punteggio secondo il Dutch Lipid Score e successivamente eseguita l'indagine genetica.
34 pazienti - perlopiù familiari non affetti dei probandi - avevano uno score inferiore a 5 e di questi solo 3 sono risultati positivi alla ricerca di mutazioni nel gene (8,8%). A 12 pazienti era stato attribuito uno score tra 6 e 8 e di questi 7 hanno ricevuto una conferma al test genetico (58%). Infine, 42 soggetti avevano un punteggio superiore a 8 e il 100% è risultato essere FH, compresi tre omozigoti ed un eterozigote composto.
La mutazione più frequentemente riscontrata è stata FH Modena (17 soggetti), seguita da FH Napoli 7 e FH Palermo. Segnalate inoltre due mutazioni non precedentemente note nel laboratorio di Modena: c.93_99 nell'esone 2 e Trp541Cys, quest'ultima presente in 5 soggetti appartenenti a due famiglie non consanguinee.
I nostri dati preliminari confermano pertanto la validità del Dutch Lipid Score nella stratificazione del rischio di FH sulla base dei dati clinici; in particolare, ai soggetti con score >8 potrebbe essere posta diagnosi senza dover ricorrere alla conferma genetica.
Tuttavia, la possibilità di ricercare direttamente le mutazioni mantiene una certa importanza in quanto permette di diagnosticare i casi di omozigosi o eterozigosi composta, di identificare le mutazioni maggiormente frequenti nella popolazione, di definire il rischio di ricorrenza nella progenie e individuare i casi che necessitano di monitoraggio più attento e trattamento più intensivo e precoce.

ELEVATED SERUM URIC ACID LEVELS IN PATIENTS WITH NAFLD: IMPLICATIONS FOR CARDIOVASCULAR RISK

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Background. Elevated serum levels of uric acid are commonly associated with both increased cardiovascular risk and elevated prevalence of non-alcoholic fatty liver disease (NAFLD).
Aims. The aim of the study was to investigate the association between cardiovascular risk, as assessed by the Framingham risk score (FRS), and serum levels of serum uric acid in a population with cardio-metabolic risk factors screened for the presence of NAFLD.
Materials and Methods. 466 consecutive subjects, referred for suspected metabolic disease, were enrolled; in 351 patients NAFLD was diagnosed by ultrasound according to Hamaguchi's criteria. Routine laboratory evaluations were performed for each patient and anamnestic and anthropometric data were collected. Cardiovascular risk was assessed using the FRS which takes into account age, gender, total cholesterol, HDL cholesterol, smoking habits and systolic blood pressure.
Results. Mean age was 55,4±13 ys in patient with NAFLD and 53,9±11,6 in patients without (P>0,05). Women were 35,9% and 39,6%, respectively; prevalence of metabolic syndrome was 66,6% and 33,7%, (P<0,001). Diabetes prevalence was 29,6% in NAFLD patients and 15,6% in patients without (P<0,001). HDL was lower in NAFLD group (47,2±14,2 vs 54,6±15,1 mg/dl; P<0,001) while uric acid levels were significantly higher (5,9±1,2 vs 5,0±1.1 mg/dl; p<0,001). Mean FRS in the whole population was 6% and there were no differences among the two groups.
The prevalence of NAFLD increased progressively across tertiles of serum uric acid levels (68,1% in first, 83,4% in second and 91,9% in third tertile, p<0,001). In addition, mean FRS increased progressively across uric acid tertiles (3%, 6% and 8% respectively p<0,001). A multiple regression analysis showed that FRS (B:0,035 P<0,001) and NAFLD prevalence (B:0,573 P<0,001) were independently associated with uric acid levels. In NAFLD patients, a strong linear correlation between FRS and serum uric acid was observed (r=0,26, p<0.001).
Conclusions. Our data confirmed increased uric acid levels in patients with NAFLD compared to controls. Moreover, an independent association between serum uric acid levels and FRS has been observed. These findings suggest that serum uric acid might contribute to improve cardiovascular risk stratification in patients with NAFLD.

THE RELATION OF SERUM LIPOPROTEIN (A) CONCENTRATION AND APOLIPOPROTEIN (A) PHENOTYPE WITH PRECLINICAL ATHEROSCLEROSIS

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Lipoprotein (a) [Lp(a)] is a plasmatic lipoprotein, comprising a low density lipoprotein-like particle with the addition of an apolipoprotein (a) molecule, covalently bonded to apolipoprotein B100.
High levels of Lp(a) have been linked to an increased risk of cardiovascular events. Lp(a) levels are mostly attributed to genetic determinants within the apolipoprotein(a) gene (LPA). We examined whether Lp(a) plasma levels, isoforms and polymorphisms are associated with early atherosclerosis by measuring intima-media thickness (IMT) in an asymptomatic population. We assessed data for 60 adult subjects from our Lipid Clinic, in primary prevention and with Lp(a) levels >35 mg/dL. We collect data for Lp(a) concentrations in plasma, LPA kringle IV type 2 (KIV-2) sums of repeats (affecting isoform size), and carrier status for the LPA single-nucleotide polymorphisms (SNP) rs10455872 and rs3798220. Lp(a) was quantified by ELISA test and isoform size by electrophoresis (SDS-PAGE). Isoforms were classified as F, B, S1, S2, S3, S4 based on electrophoretic mobility compared with apoB100. LPA genotype was assessed using a Human CVD BeadChip.
Lp(a) mean plasma level was 117 mg/dL (range 36-375 mg/dL). Small isoform apo(a) size (F,B,S1,S2) was present in the 80% of subjects and confirm the inverse relation with plasmatic Lp(a) concentration. Genetic analysis revealed that 26% of subjects carries SNP rs10455872, while 17% carries rs3798220, which are associated with increased Lp(a) levels in carriers versus non-carriers.
Finally the carotid IMTs were determined to analyze the association of Lp(a) and with IMT and we found that IMT increased with increase in quartiles of IMT ($p<0.05$) confirming a relationship between Lp(a) and development of atherosclerosis.

PLASMA HOMOCYSTEINE DISTRIBUTION IN A POPULATION OF CHILDREN WITH HYPERCHOLESTEROLEMIA

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Aim. increased plasma homocysteine levels are considered an independent risk factor for cardiovascular disease (CVD) in adults. Aim of this study is to evaluate plasma homocysteine levels in a population of hypercholesterolemic children and its correlation with positive family history for CVD, severe hypercholesterolemia, pubertal stage and MTHFR polymorphism.
Methods. 186 severely hypercholesterolemic children (median age 8,7y, 80 male/106 female) were evaluated for: family history for CVD, anthropometric measures, pubertal stage (Tanner =1 vs >1), lipid profile (enzimatic method), total plasma homocysteine (tHcy) levels, folic acid and vitamin B12 levels, (electhrochemiluminescence) and MTHFR polymorphism by polymerase-chain-reaction.
None was on pharmacological treatment or vitamin supplementation.
Statistics. Student's t test or Mann-Whitney test for independent samples.
Results. The 186 patients were divided in two groups: 90 (48%) had a family history for premature CVD (CVD+), 96 (52%) only for hypercholesterolemia (CVD-). Blood lipid profile was not different by age, sex, pubertal stage. Plasma tHcy levels were not statistically different: Hcy (umol/l, mean±ds): 5.87±3.37, 5.46±1.82. Serum folate and vitamin B12 levels were within normal range.
tHcy levels were significantly higher in pubertal (Tanner >1, 41 children) than in pre-pubertal stage (Tanner =1, 145 children), respectively 6.49±4.30 vs 5.37±1.83 (p=0,016). Considering MTHFR C677T polymorphism (genotype CC, CT and TT). Not statistically difference was noticed in tHcy, folate and vitamine B12 plasma levels within the three groups in pre-pubertal children (Tanner =1); Hcy (umol/l, mean±ds): 5.42±1.65, 5.22±1.66, 5.58±2.41.
Children with MTHFR genotype CT or TT and Tanner >1 had higher tHcy plasma levels (respectively 6.76±5.37 in CT and in 8.04±5.30 in TT), and folate plasma levels at the lower end.
Conclusions. In our population we did not find any correlation between tHcy levels and family history for CVD. tHcy levels seem to relate with pubertal activation. Molecular analysis for MTHFR could be useful in pediatric population to detect both homozygous and heterozygous subjects, who could benefit from vitamin supplementation to prevent moderate hyperhomocysteinemia and CVD in adulthood.

ASSOCIAZIONE TRA IPERURICEMIA ED AUMENTATA PREVALENZA DI FIBRILLAZIONE ATRIALE IN PAZIENTI OSPEDALIZZATI CON DIABETE MELLITO TIPO 2

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Background e Scopo. L'iperuricemia e la fibrillazione atriale (FA) sono due condizioni patologiche molto comuni nei pazienti affetti da diabete mellito tipo 2 (T2DM) e condividono molteplici fattori di rischio cardiovascolare. Attualmente, esistono pochi dati in letteratura sulla possibile relazione tra elevati livelli circolanti di acido urico e rischio di FA nei pazienti affetti da T2DM.
Metodi. In questo studio abbiamo arruolato tutti i pazienti ospedalizzati con T2DM, che sono stati dimessi dalla Divisione di Endocrinologia della AOUI di Verona nel periodo 2007-2011. Nel caso che un paziente avesse avuto molteplici ricoveri, tale paziente veniva conteggiato un'unica volta e, per l'analisi statistica, venivano utilizzati solo i dati del ricovero che avesse informazioni complete. Globalmente, nel database sono stati identificati 867 pazienti con T2DM. Dopo esclusione dei pazienti con neoplasia e di quelli in cui non era disponibile il dato dell'uricemia, 842 pazienti (M/F=463/379; età media 69 anni) sono stati inclusi nell'analisi statistica. La presenza di iperuricemia era definita come valori circolanti di acido urico >7 mg/dl per i maschi e >6 mg/dl per le femmine e/o trattamento con allopurinolo. La diagnosi di FA permanente/persistente era confermata nei pazienti affetti da cardiologi esperti mediante la storia clinica e l'esecuzione di ECG durante la degenza ospedaliera.

Risultati. Degli 842 pazienti diabetici ospedalizzati inclusi nello studio, 243 (28.9%) avevano iperuricemia e 91 (10.8%) erano affetti da FA persistente o permanente. Nessun paziente aveva FA parossistica né storia clinica di gotta. I pazienti con iperuricemia (n=243) avevano una prevalenza di FA permanente/persistente che era marcatamente più elevata (20.6% vs 7.1%; P<0.001) rispetto ai pazienti con normali valori di acido urico (n=599). Nell'analisi di regressione logistica l'iperuricemia si associava alla presenza di FA (odds ratio 3.41, 95% CI 2.19-5.32; P<0.001). Tale associazione rimaneva statisticamente significativa anche dopo aggiustamento per età, sesso, fumo, emoglobina glicata, ipertensione arteriosa (definita come PA≥140/90 mmHg e/o uso di farmaci anti-ipertensivi, incluso diuretici), malattia renale cronica (definita come eGFR <60 ml/min/1.73 m^2 e/o macroalbuminuria), precedente storia di cardiopatia ischemica, cardiopatia valvolare, BPCO, ipertiroidismo ed uso di digitale, anti-aggreganti ed anticoagulanti (adjusted-odds ratio 5.63, 95%CI 1.33-23.8; P=0.01).
Conclusioni. Questo è il primo studio osservazionale che documenta la presenza di una significativa associazione fra iperuricemia asintomatica ed aumentata prevalenza di FA in un ampio campione di pazienti ospedalizzati affetti da T2DM. Tale associazione rimaneva significativa anche dopo aggiustamento per un ampio numero di possibili fattori confondenti. Sono necessari, ovviamente, ulteriori studi per confermare tali risultati e per valutare i possibili meccanismi eziopatogenetici che possono spiegare tale associazione.

IN VITRO EFFECTS OF METABOLITES OF ELLAGITANNINS ON EARLY STEPS OF ATHEROGENESIS

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Background and Aim. Ellagitannins are a subclass of tannins found in red fruits and oak-aged wines. They have anti atherosclerotic activity in vitro, but these compounds are poorly absorbed by intestine after oral ingestion, since they are metabolized by gut microbiota, producing catabolites called urolithins (uro D, C, A, B). Therefore this study aimed to evaluate urolithin effects on cholesterol metabolism and on endothelial function in early stage of atherogenesis. During these key-events, an increased expression of cell adhesion molecules (ICAM-1,VCAM-1) occurs, leading to monocyte adhesion into the intima and, after LDL uptake, conversion in lipid-rich foam cells.
Methods. Cholesterol efflux: THP-1 macrophages were incubated with radiolabelled cholesterol. Successively they were exposed to urolithins (5-10 μM) and cholesterol acceptors (human serum,1%v/v). Afterwards cholesterol was quantified by a radioisotope assay.
Cholesterol loading: cholesterol-enriched THP-1 macrophages were treated with urolithins (1-5-10 μM). After 24 h, cells were lysed and intracellular cholesterol quantified by fluorimetric analysis. Monocyte adhesion to endothelial cells: HUVECs were treated with urolithins (10 μM) and TNF-α (15 ng/ml) and, after 30 minutes of incubation with calcein-labelled THP-1 monocytes, fluorescence intensity was measured.
SolubleVCAM secretion from HUVECs: sVCAM were quantified using ELISA-kit in the supernatant of endothelial cells treated with urolithins (5-10 μM) and TNF-α (15 ng/ml).
Results. Urolithin C significantly improved cholesterol efflux, while Urolithin A+B slightly increase it. In addition, Urolithin C reduced cholesterol accumulation (-21,3%, p<0,01), while urolithin A+B inhibits cholesterol uptake (29,3%, p<0.001). Ellagic acid and urolithin C significantly reduced sVCAM production (-26,5%, p<0.05 and -17,5%, p<0.001 respectively). Finally, all compounds significantly inhibited monocyte adhesion to endothelial cells.
Conclusions. In this work, we observed a potential activity of urolithins on different stages of atherogenesis. They inhibit monocyte adhesion to endothelium; and the association Urolithin A+B and Urolithin C reduced significantly cholesterol uptake. Current studies may provide mechanistic explanations of Ellagitannins effects on atherogenesis and in vivo cardiovascular protective capacity.

SPLEEN DIMENSIONS EVALUATED BY ULTRASOUND ARE INVERSELY ASSOCIATED WITH LYSOSOMAL ACID LIPASE ACTIVITY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aim. Nonalcoholic fatty liver disease (NAFLD) represents the most common form of chronic liver disease worldwide. Mechanisms involved in NAFLD onset and progression are not fully explained. Lysosomal Acid Lipase (LAL) is a key enzyme in lipid metabolism and a reduction of its activity may contribute to intracellular lipid accumulation in adult NAFLD. LAL deficiency is a rare autosomal recessive genetic disease. Two main different phenotypes may be present: Wolman Syndrome, rapidly fatal, and Cholesterol Ester Storage Disease (CESD), a late onset phenotype that occurs with fatty liver, elevated aminotransferase levels, dyslipidaemia, hepatomegaly and splenomegaly. LAL deficiency has been suggested as an under-recognized cause of NAFLD but it is not clear in which patients it would be useful to test LAL activity. Ultrasonography (US) determination of spleen dimensions is accurate and easy to perform. The aim of this study was to determine if spleen biometry evaluated by US is associated with LAL activity in a population of NAFLD patients.
Methods. The study has been performed in 172 consecutive patients referred for suspected metabolic disease and with a liver US scanning positive for NAFLD. Spleen longitudinal diameter and spleen area were measured during the abdominal ultrasound exam. Splenomegaly was defined as a spleen area >45 cm^2. Liver steatosis severity was defined according to Hamaguchi US criteria. All subjects underwent routine clinical and biochemical evaluation. LAL activity was measured with dried blood spot method (Lalistat2, nmol/spot/h) in all patients.
Results. Mean age was 55,4±13 years and 35,9% of patients were women. Prevalence of metabolic syndrome an diabetes were 66,6% and 33,7%, respectively. Stratifying population in LAL quartiles, a significant decrease of spleen area from the lower to the highest quartile was found (37.6±10.08 vs 36.67±12.90 vs 34.49±10.07 vs 31.52±8.23; p<0.05). Linear bivariate regression confirmed an inverse correlation between LAL activity and spleen diameter (r=-0.157; p<0.028), and spleen area (r=-0.23; p<0.002). Splenomegaly was present in 5.7% of patients. LAL activity was significantly reduced in subjects with splenomegaly (0.75 vs 0.88 nmol/spot/h; p=0.04). In a stepwise multiple linear regression analysis, spleen area (p=0.003) and total cholesterol (p=0.023) were inversely associated with values of LAL activity.

Conclusion. Our data show an inversely correlation between spleen dimensions evaluated by US and LAL activity in a population of NAFLD patients. LAL activity is significantly reduced in patients with splenomegaly than in those without. Evaluation of spleen dimensions could help to identify patients to test for LAL activity.

LA SIMVASTATINA INDUCE L'AUMENTO DELLA ESCREZIONE URINARIA DI AQUAPORINA-2 (UAQP2) IN PAZIENTI IPERCOLESTEROLEMICI. IMPLICAZIONI TERAPEUTICHE DURANTE ALTERATO TRAFFICO DI UAQP2

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Un effetto pleiotropico inedito delle statine è quello di aumentare l'espressione in membrana plasmatica del canale per l'acqua AQP2 nel dotto collettore renale nei roditori, potenziando così la capacità di concentrare le urine (1). Effetto mai stato studiato nell'uomo e potenzialmente utile per il trattamento di difetti di concentrazione dell'urina. Sono stati arruolati 24 pazienti ipercolesterolemici näive che hanno iniziato trattamento con simvastatina 20 mg/die, o con monacolina K (=lovastatina, 10 mg/die) o sola dieta ipolipidica dosando l'escrezione urinaria di uAQP2 al baseline e per 12 settimane. Un gruppo di 13 pazienti, già in trattamento cronico con statine, è servito come controllo. Tutte le terapie hanno significativamente e progressivamente ridotto il colesterolo plasmatico dei pazienti naïve. Solo il trattamento con simvastatina ha però incrementato i livelli di uAQP2: del 53% rispetto al basale già dopo una settimana di trattamento, e del 65% rispetto al basale dopo 12 settimane di trattamento. In linea con questo effetto, anche la capacità di concentrazione delle urine in questi pazienti è stata influenzata dal trattamento con simvastatina. Alla fine del trattamento, la diuresi si riduceva del 15% rispetto al basale e l'osmolalità urinaria incrementava del 54%. Né la monacolina K, né la dieta ipolipidica hanno modificato l'uAQP2 o i parametri urinari. È interessante notare che l'uAQP2, la diuresi e l'osmolalità urinaria dei pazienti naïve, alla fine del trattamento con simvastatina, non erano significativamente differenti da quelli misurati nei pazienti già cronicamente trattati con statine, suggerendo che questo effetto pleiotropico delle statine nel rene perdura nel tempo.
Per la prima volta si dimostra che le statine potrebbero migliorare l'efficacia dell'attuale trattamento farmacologico di difetti di concentrazione dell'urina come il diabete insipido nefrogeno legato all'X (X-NDI).

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HIGH DENSITY LIPOPROTEIN (HDL)-ASSOCIATED SPHINGOSINE 1-PHOSPHATE (S1P) INHIBITS MACROPHAGE APOPTOSIS BY STIMULATING SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) ACTIVITY AND SURVIVIN EXPRESSION

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Background and Aims. Macrophage apoptosis is a critical process involved in atherosclerosis. High density lipoprotein (HDL) carries biologically active lipids such as sphingosine-1-phosphate (S1P) that may contribute to its atheroprotective capacity. We here examined the effect of S1P and HDL on the apoptosis of RAW264.7 murine macrophages.
Methods and Results. Mitochondrial or endoplasmic reticulum-dependent apoptosis was induced by exposure of RAW264.7 cells to etoposide or thapsigargin, respectively. Cell death induced by each of these compounds was inhibited by S1P as assessed by annexin V binding and TUNEL staining. In addition, activation of both terminal caspase 3 and upstream caspases 9 and 12 were inhibited in RAW264.7 cells pretreated with S1P. S1P induced expression of the inhibitor of apoptosis protein (IAP) family proteins cIAP1, cIAP2 and survivin, which inhibit caspase activity, but only the suppressant of survivin expression YM155 and not the cIAP1/2 blocker GDC0152 reversed the inhibitory effect of S1P on macrophage apoptosis. We further observed that S1P activated signal transducer and activator of transcription 3 (STAT3) and Janus kinase 2 (JAK2) and the stimulatory effect of S1P on survivin expression and its inhibitory effect on apoptosis were attenuated by STAT3 or JAK2 inhibitors, S3I-201 or AG490, respectively. The effects of S1P on STAT3 activation, survivin expression and apoptosis were emulated by HDL and HDL lipids, but not by HDL deprived of S1P by incubation with charcoal. In addition, JTE013 and CAY10444, S1P receptor 2 and 3 antagonists, respectively, compromised the capacity of S1P and HDL to stimulate STAT3 activation and survivin expression, and to inhibit apoptosis.
Conclusions. HDL-associated S1P inhibits macrophage apoptosis by stimulating STAT3 activity and survivin expression. The suppression of macrophage apoptosis may represent a novel mechanism utilized by HDL to exert its anti-atherogenic potential.

FAMILIAL CHYLOMICRONEMIA AND GPIHBP1 GENE MUTATIONS: ANOTHER PIECE OF THE PUZZLE.

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Background. The intravascular lipolysis of Triglyceride(TG)-rich lipoproteins mediated by Lipoprotein Lipase (LPL) requires some co-factors including:
1) apolipoprotein CII and apolipoprotein A-V, LPL activators;
2) GPIHBP1 protein, a molecular platform on endothelial cells for LPL lipoproteins interaction;
3) LMF1, a peptide required for the maturation of LPL in LPL secreting cells.

Severe hypertriglyceridemia with chylomicronemia (TG>10 mmol/L) may occur if LPL or one of the co-factors is defective.
Methods. We sequenced the lipolysis genes in patients with severe Hypertriglyceridemia without mutations in LPL gene.
Results. We identified four patients found to carry two mutant GPIHBP1 gene alleles. Their fasting plasma TG level ranged from 13 to 18 mmol/L. Three patients had a positive history of recurrent pancreatitis. Patient #1 was a compound heterozygote for a minute insertion/deletion (which eliminated the canonical termination codon in mRNA) and a missense mutation (p.Ser107Pro) predicted in silico to be damaging. Patient #2 was homozygote for the missense mutation p.Cys83Arg which disrupts the structure of Ly6 domain of GPIHBP1 protein and abolishes LPL binding. Patient #3 was homozygote for a G>T transition in exon 1 which converts the ATG methionine initiation codon into ATT isoleucine codon, thus preventing translation initiation1. Patient #4 was homozygote for the missense mutation p.Thr80Lys, which had previously been found to abolish the binding LPL1. The survey of the literature revealed that up to now 23 mutations of GPIHBP1 gene were found worldwide in patients with familial chylomicronemia.
Conclusions. Mutations in GPIHBP1 gene in homozygous/compound heterozygote state may cause an impairment of the LPL-mediated intravascular lipolysis of TG-rich lipoproteins. Defects of GPIHBP1 function should be considered as a cause of severe hypertriglyceridemia in patients without LPL mutations.

SUPPRESSOR OF CYTOKINE SIGNALING-3 (SOCS-3) INDUCES PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 (PCSK9) EXPRESSION IN HEPATIC HEPG2 CELL LINE

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The suppressor of cytokine signaling (SOCS) proteins are negative regulators of the JAK/STAT pathway activated by pro-inflammatory cytokines, including the tumor necrosis factor-α (TNF-α). SOCS3 is also implicated in hypertriglyceridemia associated to insulin-resistance (IR).
Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) levels are frequently found to be positively correlated to IR and plasma very low-density lipoprotein-triglycerides (VLDL-TG) concentrations. The present study aimed to investigate the possible role of TNF-α and JAK/STAT pathway on *de novo* lipogenesis and PCSK9 expression in HepG2 cells. TNF-α induced both SOCS3 and PCSK9 in a concentration-dependent manner.
This effect was inhibited by transfection with siRNA anti-STAT3, suggesting the involvement of the JAK/STAT pathway. Retroviral overexpression of SOCS3 in HepG2 cells ($HepG2^{SOCS3}$) strongly inhibited STAT3 phosphorylation and induced PCSK9 mRNA and protein, with no effect on its promoter activity. Consistently, siRNA anti-SOCS3 reduced PCSK9 mRNA levels while an opposite effect was observed with siRNA anti-STAT3. In addition, $HepG2^{SOCS3}$ express higher mRNA levels of key enzymes involved in the *de novo* lipogenesis, such as fatty-acid synthase (FAS), stearoyl-CoA desaturase, and apo-B.
These responses were associated with significant increase of SCD-1 protein, activation of SREBP-1, accumulation of cellular TG, and secretion of apoB. $HepG2^{SOCS3}$ shows lower phosphorylation levels of IRS-1 Tyr^{896} and Akt Ser^{473} in response to insulin. Finally, insulin stimulation produced an additive effect with SOCS3 overexpression, further inducing PCSK9, SREBP-1, FAS and apoB mRNA. In conclusion, our data candidate PCSK9 as a gene involved in lipid metabolism regulated by pro-inflammatory cytokine TNF-α, in a SOCS3 dependent manner.

EFFETTI DELLA CHIRURGIA BARIATRICA SUL RISCHIO CARDIOVASCOLARE IN PAZIENTI DIABETICI. GRUPPO INTERDISCIPLINARE DI CHIRURGIA DELL'OBESITÀ DI VERONA

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Introduzione. La chirurgia bariatrica (CB) è il mezzo più efficace per ottenere e mantenere il calo ponderale e indurre la remissione del diabete tipo 2 (DM2). Il rischio cardiovascolare (RCV) del paziente obeso è mediato da alterazioni metaboliche e comorbidità che presentano un sostanziale miglioramento dopo calo ponderale CB-indotto.
Scopo. Valutare l'effetto della CB sul RCV di pazienti obesi-diabetici.
Metodi. Sono stati studiati 97 pazienti obesi con DMT2 (durata diabete 5.7±5.6 anni); 59 donne e 38 uomini, età 49.7±8.6 anni, BMI 46.2±7.7 kg/m^2, sottoposti a bendaggio gastrico (LAGB,N=10), o bypass gastrico (RYBP,N=70), o sleeve gastrectomy (SG,N=17) nel periodo 2005-2012. In tutti i pazienti sono stati valutati al basale e dopo un anno dall'intervento parametri antropometrici, pressione arteriosa, glicemia, emoglobina glicata, profilo lipidico, abitudine tabagica. Lo score di RCV è stato valutato al basale e dopo un anno utilizzando lo score UKPDS, lo score progetto cuore (PC) e l'algoritmo di Framinghan (F).
Risultati. Il calo ponderale medio a 12 mesi dall'intervento è stato del 26.4±9.1% e la remissione del Diabete si è verificata nel 69% dei soggetti (4/10 dei LAGB, 46/70 di RYBP e 14/17 di SG). Ad un anno dall'intervento in tutti i pazienti si è osservato un miglioramento dello score-RCV (deltaUKPDS=-3.6±4.3; deltaPC=-2.4±3.1; deltaF=-1.4±3.6) senza differenze significative per intervento chirurgico eseguito. I tre score-RCV erano significativamente correlati (Rho di Spearman): UKPDS base e PCbase: 0.86, p<0.001; UKPDS base e F base: 0.80, p<0.001; PC e F base: 0.87, p<0.001
Conclusioni. Il calo ponderale ottenuto con la CB in pazienti diabetici con grave obesità e ad elevato rischio cardiovascolare, determina un miglioramento del profilo di RCV. I 3 score di RCV utilizzati descrivono tale fenomeno in modo paragonabile.

LIPOPROTEINA(A): UN MARKER DI RISCHIO VASCOLARE QUALE MARKER DI COMPLICANZE IN GRAVIDANZA

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Introduzione. Una storia di complicanze gravidiche placenta mediate è associata ad un incremento del rischio cardiovascolare futuro. La Lipoproteina(a) [Lp(a)], fattore di rischio cardiovascolare, è stata scarsamente investigata nella patologia ostetrica. In questo studio abbiamo valutato i livelli di Lp(a) in donne con storia di eventi ostetrici avversi (PMPC) comparate con donne con storia di gravidanza fisiologica (HW).
Materiali e Metodi. La Lp(a) è stata determinata in 360 donne con storia di PMPC [154 preeclampsia (PE), 121 morte fetale (SB) e 85 ritardo di crescita (SGA)] comparate con 270 HW.
Risultati. I livelli di Lp(a) sono risultati significativamente più elevati nelle donne con storia di PMPC rispetto a quanto osservato nelle HW (p=0.03), e risultavano associati ad un incremento del rischio di PMPC [OR=1.93 (1.20-3.09), p=0.006], anche dopo aggiustamento per età, familiarità per patologia cardiovascolare e fattori di rischio tradizionali. Analizzando i livelli di Lp(a) in relazione al tipo di complicanza gravidica, abbiamo osservato che donne con storia di PE e SB presentavano livelli significativamente più elevati di Lp(a) rispetto a quanto osservato nelle HW (p=0.05 e p=0.03, rispettivamente), mentre nelle donne con storia SGA i livelli di Lp(a) sono risultati superiori, anche se non significativamente, rispetto a quanto osservato nelle HW. Analizzando l'associazione tra Lp(a) ed il rischio di ciascun evento ostetrico avverso, abbiamo osservato che livelli elevati di Lp(a) incrementavano il rischio di PE e di SB rispettivamente di 2 e 2.5 volte, anche dopo aggiustamento.
Questo studio evidenzia per la prima volta il ruolo della Lp(a) nel rischio di SB, e confermano l'associazione tra elevate concentrazioni di Lp(a) e la storia di PE; in donne, portatrici di un nuovo fattore di rischio CV, come la storia ostetrica, elevati livelli di Lp(a), noto marcatore aterotrombotico, potrebbero permettere di identificare giovani donne a più elevato rischio CV futuro.

EVALUATION OF THE HEALTH BENEFITS OF SOY PROTEIN CONSUMPTION IN PATIENTS CARRYING THE METABOLIC SYNDROME

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Introduction. Circulating lipids are a modifiable risk factor for cardiovascular disease (CVD) and their improvement results in CVD risk prevention. Drug treatment is widely used in selected high-CVD risk patients, although dietary management of hyperlipidemias remains the main approach to reduce cholesterolemia and CVD risk. The use of vegetable proteins, namely soy proteins, offered positive results, providing a rationale for their use in the dietary management of individuals with borderline-high total and LDL-cholesterol and at risk of CVD.
Aim. To evaluate the effect of a daily intake of 30 g soy protein on lipid parameters, visceral adipose accumulation, and adipokine levels.
Study design. Randomized, parallel, single-centre study, intervention duration: 12 weeks. Patients: Sixty-six (32 M/34 F) patients with mild metabolic syndrome (3/5 NCEP-ATPIII criteria) were randomly allocated to either control diet (hypolipidic diet containing protein from animal sources; N=28; 15 M/13 F) or to soy diet (hypolipidic diet containing 30 g/day soy protein; N=36; 17 M/19 F).
Results. Dietary soy intervention, compared to control diet, resulted in a significant reduction of total cholesterol (-6.7%, p=0.001), LDL-C (-6.7%, p=0.013) and apoB (-13.5%, p=0.002) levels. Total HDL-C and their subclasses did not change. Compared to control group, patients at soy showed significantly decreased body

weight (-2.1%, p=0.015) and BMI (-2.2%, p=0.019). Waist circumference was reduced in both arms. Plasma leptin was reduced by -18.2% (p=0.03) in the soy group, vs. controls, but adiponectin was not affected in either arm, leading to a significant decrease of the leptin:adiponectin ratio only in patients at soy. A same trend was shown for insulin (-4.9%, p=0.05), HOMA-IR (-11,6%, p=0.03) and sICAM-1 (-5.3%, p=0.007).
Conclusion. The results of the present study highlight the substantial safety and elevated compliance of this intervention, which resulted in a significant improvement of a series of biomarkers associated to primary CVD risk.

THE PROFILE OF PATIENTS WITH METABOLIC SYNDROME ON FIXED DOSE COMBINATION DRUGS

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Background and Aim. Role of fixed dose combination (FDC) therapy in patients affected by Metabolic Syndrome (MS) is still debated.
Therefore we have analyzed the profile of MS patients treated or not with FDC in association to other drugs.
Methods. Retrospective evaluation of 454 subjects consecutively coopted during the last 2 years on suspicion of MS: 315 were <65 yrs (M/F=162/153, mean age 51), 138 >65 yrs (M/F=56/82, mean age 72).
A complete clinical, echocardiographic, ultrasonographic and biological evaluation was carried out.
Results. Roughly, among all subjects, 21% is not on drug therapy but only on lifestyle correction prescription due to milder forms of MS; 16.7% received only one drug, over 60% was on multidrug therapy.
132 subjects (29,1%) were on FDC, mainly ACE-inhibitors or ARBs with hydrochlorothiazide (respectively 59.1% and 25.7%), lower rates of other fixed combinations of antihypertensive drugs; 10% assumes FDC of oral antidiabetics.
In comparison to the remnant patients, those on FDC resulted to have statistically significant differences for: age (62,3+10.2 vs 55.2+13.3, p=0.00001), Framingham CV risk (14,9+10,9 vs 12,2+11,1, p=0.002), values of systolic blood pressure (138,1+16,2 vs 133,4+15,7, p=0.002), blood glucose levels (121,5+39,4 vs 114,7+40,8, p=0.003), xAST values (1,2+7,3 vs 0,7+0,4, p=0.005), US preperitoneal fat (mm) (13,5+14,1 vs 14,1+11,3, p=0.04).
No significant differences were ruled out for lipemic profile and insulin resistance or microalbuminuria.
Conclusion. The profile of patients on FDC affected by MS is characterized by an older age, higher CV risk, elevated values of SBP and blood glucose level as well as higher severity of "minor" comorbidities of MS such as steatosis and fat distribution.

LA TERAPIA CRONICA CON LDL AFERESI MIGLIORA IL FLUSSO CORONARICO MISURATO DURANTE ECO STRESS CON DIPIRIDAMOLO

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Scopo dello studio. Gli effetti benefici dell'LDL aferesi (LA) sull'omeostasi cardiovascolare, le funzioni dell' endotelio e il microcircolo sono noti sin dagli anni '90. Ad oggi, la misura quantitativa del flusso coronarico è ancora affidato alla PET con ammoniaca che non è agevole strumento per un follow-up a lungo termine che è, invece indicato nei soggetti con cardiopatia ischemica.
Lo scopo del nostro studio è stato quello di analizzare l'andamento temporale della riserva di flusso coronarico (CFR) su arteria discendente anteriore durante ecocardiogramma da stress con dipiridamolo in pazienti in trattamento cronico di LA per ipercolesterolemia familiare "refrattaria" già complicata da cardiopatia ischemica.
Materiali e Metodi. Durante il periodo in esame 10 pazienti (età media 65±7 anni, maschi 70%) affetti da ipercolesterolemia familiare (6/10 FH, 4/10 FCH) in terapia ipolipemizzante massimale, con storia di malattia cardiovascolare sono stati sottoposti a LA presso il nostro Centro con cadenza bisettimanale (sistema Liposorber®-LA systems; Kaneka, Osaka, Japan o HELP®, Plasmat-Futura®; B|Braun, Melsungen, Germany in accordo con le caratteristiche cliniche del paziente). Elevati livelli di Lp"a" (>60 mg/dl) erano presenti in 6/10 soggetti. Questi paziente erano cronicamente trattati con LA da 7 anni (range interquartile 6-14 anni). La malattia coronarica era stata diagnostica ad un'età media di 44±8 anni. Non rilevanti comorbidità erano presenti. Tutti i pazienti, in relazione alla malattia coronarica, e secondo le indicazioni delle linee guida, eseguivano ecocardiogramma da stress farmacologico con dipiridamolo non modificando la terapia anti-ischemica in atto durante tutto il periodo di osservazione.
La CFR su arteria discendente anteriore è calcolata come rapporto tra la velocità diastolica campionata al picco dello stress su valore campionato basalmente (v.n >2.0). Questo parametro quantitativo, complementare alla valutazione della cinetica del ventricolo sinistro durante ecocardiogramma da stress farmacologico, aggiunge capacità di stratificazione prognostica all'ecostress sia in paziente con che senza terapia anti-ischemica.
Risultati. Durante il follow-up mediano di 26 mesi [19-36 mesi] abbiamo rilevato un significativo incremento della CFR su arteria discendente anteriore (da 1.86±0.47 a 2.39±0.42 - p=0.011, t-test per dati appaiati) con ecostress negativo per difetti di cinetica regionale indotti da dipiridamolo. Durante il periodo in studio non sono state effettuate variazioni della terapia cardio-attiva e non si sono verificati eventi cardiovascolari.
Conclusioni. La CFR durante ecocardiogramma da stress con dipiridamolo è uno strumento affidabile nel follow-up di soggetti con ipercolesterolemia familiare severa. L'aumento della CFR nel tempo è indicativo di una migliore perfusione coronarica, target primario di misura dell'efficacia della terapia ipocolesterolemizzante con LDL aferesi.

SCREENING DELLE IPERCOLESTEROLEMIE FAMILIARI: IL RUOLO DELLA MEDICINA DEL LAVORO

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Premessa e Scopo dello studio. L'ipercolesterolemia autosomica dominante (ADH) è una delle malattie monogeniche più frequenti ed è strettamente correlata a malattie cardiovascolari (infarto miocardico, ictus). ADH è clinicamente caratterizzata da alti livelli plasmatici di colesterolo LDL, xantomatosi tendinea, arco corneale e coronaropatia in età precoce. I geni classicamente associati con ADH sono il gene del recettore LDL (ipercolesterolemia familiare o FH) e Apo B gene (Detective familiare ApoB o FDB). Negli ultimi anni sono state descritte due mutazioni del gene PCSK9, responsabile della terza forma di ADH (FH3). La frequenza di FH in eterozigosi è stimata essere di circa 1/500 nella popolazione generale; per tael motivo, questa malattia è una delle sindromi monogeniche più comuni. ADH è scarsamente sintomatica in fase preclinica, e ancora oggi, in un certo numero di casi, la diagnosi viene effettuata soltanto dopo un evento vascolare acuto. Pertanto, lo scopo di questo studio è valutare il ruolo dello screening lipidico nella diagnosi precoce di ADH.
Materiali e Metodi. Da gennaio 2014, sono stati studiati 656 soggetti. I dati sono stati estrapolati dai dipendenti del P.O. Garibaldi di Catania, in collaborazione con la Divisione di Medicina del Lavoro. Abbiamo valutato il profilo lipidico di ogni soggetto e abbiamo escluso ipercolesterolemie secondarie attraverso esami ematochimici di secondo livello. La possibilità di ipercolesterolemia familiare è stata valutata mediante il Dutch Clinic Lipid Network score; un punteggio 3-5 (colesterolo LDL>190 mg/dl) **rendeva** possibile tale diagnosi. Inoltre, in un sottogruppo della popolazione studiata (16 soggetti), abbiamo effettuato l'analisi genetica per la ricerca di mutazioni genetiche responsabili di ipercolesterolemia familiare, all' interno del network LIPIGEN della Società Italiana per lo Studio di aterosclerosi (SISA).
Risultati. 93 soggetti della popolazione in esame (14%) aveva valori di Col. LDL >190 mg/dl parametro necessario per avere il sospetto di ipercolesterolemia familiare. All'interno del sottogruppo, l'analisi genetica ha confermato la diagnosi clinica di ipercolesterolemia familiare in 2 soggetti (13%).
Conclusioni. Ancora oggi, molti soggetti non sanno di avere elevati livelli plasmatici di colesterolo LDL. Effettuare, in questi soggetti, una accurata prevenzione cardiovascolare, è fondamentale. In questo lavoro, abbiamo proposto una strategia di screening, tramite la valutazione del profilo lipidico ad opera della Divisione di Medicina del Lavoro, per la diagnosi precoce di ipercolesterolemia familiare.

GENETIC AND NUTRITIONAL FACTORS IN DETERMINING CIRCULATING LEVELS OF LIPOPROTEIN(A): RESULTS OF THE "MONTIGNOSO STUDY"

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Introduction. Increasing evidence shows an association between high levels of lipoprotein(a) [Lp(a)] and atherothrombotic diseases. Lp(a) is a macromolecule formed by the assembly between apolipoprotein B100 and apolipoprotein(a) [apo(a)]. The apo(a), encoded by LPA gene, is characterized by a variable number of kringle IV type 2 (KIV-2) repeats, responsible of the variation in size of apo(a) and, consequently, of circulating Lp(a) levels which have to be inversely proportional to the number of KIV-2 repeats. The number of repeats depends on a genetic polymorphism represented by duplications and deletions of two exons (exon 4 and 5) and an intron (~5 Kb). Environmental factors are considered to determinate minor phenotypic variability in Lp(a) levels.
Aims. Aim of this study was to evaluate, in the cohort of subjects of the Montignoso study, whether Lp(a) circulating levels and the main genetic determinant of Lp(a) levels, the KIV-2 repeat polymorphism of LPA gene, are associated with the presence of plaques in the coronary district and with the presence of cardiovascular and thrombotic diseases. In addition, the possible gene-environment interaction between KIV-2 LPA repeat polymorphism and adherence to the Mediterranean diet and to the weekly intake of fish in determining circulating Lp(a) levels were assessed.
Methods. In the cohort of subjects of the Montignoso Study (n=1801), of which n=476 with already available Lp(a) circulating levels, after genomic DNA extraction from peripheral blood, the number of KIV-2 LPA repeats was determined by real time PCR Taqman technology.
Results. Spearman's analysis confirmed an inverse correlation ($r=-0.193$, $p<0.0001$) between Lp(a) levels and the number of KIV-2 LPA gene repeats. The prevalence of subjects with a low number of KIV-2 LPA repeats (<9.79, median value of KIV-2 distribution) was statistically higher in subjects with coronary plaques on the common trunk (p=0.015), on the proximal left anterior descending (p=0.007) and on the proximal circumflex (p=0.015). No statistically significant difference was observed in the distribution of subjects with low or high number of repeats KIV-2 and some clinical cardiovascular events, particularly coronary artery disease and myocardial infarction. No statistically significant difference was observed in the distribution of subjects with low levels (<500 mg/L) or high levels of Lp(a) according to the presence of plaques in various coronary districts and cardiovascular disease. At the multivariate logistic regression analysis adjusted for traditional cardiovascular risk factors, a low number of KIV-2 LPA repeats showed a trend as a significant and independent risk factor for development of coronary plaques odds ratio (OR) 2.16 (95% CI 0.94-4.99), p=0.071. In subjects with high number of KIV-2 LPA repeats, an influence of the adherence to the Mediterranean diet (score ≥34) on the Lp(a) levels was observed ($p<0.049$): indeed, subjects with higher score of adherence to the Mediterranean diet showed lower levels of Lp(a). Moreover, in these subjects we observed an influence of fish intake on the Lp(a) levels although it did not reach the full statistical significance (p=0.186): subjects taking more servings of fish per week had lower Lp(a) levels.

Conclusions. We demonstrated that a low number of KIV-2 repeat of the LPA gene is associated to the presence of plaques in different districts of the coronary tree. The inverse correlation between KIV-2 LPA polymorphism and Lp(a) levels was confirmed. Only in subjects with a number of KIV-2 repeats above the median of the distribution, genetically determined to have lower Lp(a) levels, the beneficial effect of fish and Mediterranean diet can be observed.

LCAT DEFICIENCY IN PREGNANCY, A CASE REPORT

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Background. Primary LCAT Deficiency is a rare genetic disorder caused by a mutation in the LCAT gene and characterized by severe hypoalphalipoproteinemia.
Here we describe the first case report of the natural history, outcome and management challenges of LCAT deficiency during pregnancy.
Methods and Results. A 29-year-old multigravida woman initially presented with bilateral corneal clouding, greatly reduced HDL-C (3 mg/dL) and proteinuria (113.7 mg/mmol creatinine). Molecular analysis confirmed the clinical diagnosis of LCAT deficiency: she was a compound heterozygote with two LCAT gene mutations one of which is novel, c.321C>A exon 3. Fasting blood was collected at 22 weeks gestation and 14 weeks post-partum to assess possible changes during pregnancy. LCAT activity, cholesterol esterification rate (CER), both undetectable, unesterified/total cholesterol ratio (UC/TC, 0.90), and LCAT mass (3.00 µg/mL) remained identical during pregnancy and post-partum. Total cholesterol, HDL-C (6 mg/dL), phospholipids, apoA-I and apoB increased during pregnancy. Her pregnancy, from the 2nd trimester, was complicated by an hypertriglyceridaemia (613 mg/dL), which was more severe than is seen with normal physiological changes of pregnancy. Surprisingly, the level of proteinuria significantly improved during pregnancy (13.9 mg/mmol creatinine), despite stopping the ACE inhibitor, but it worsened post partum again (131.6 mg/mmol creatinine). Lipoprotein analysis by FPLC and agarose gel electrophoresis of plasma and 1.020-1.063 g/mL fraction revealed the presence of lipoprotein X (LpX) only in post-partum, whereas this abnormal particle disappeared during pregnancy.
Conclusion. LpX is an abnormal cholesterol- and phospholipid-rich lipoprotein particle, whose presence has been proposed as a major causative factor in the development of renal disease in carriers of LCAT deficiency. Its disappearance during pregnancy improves proteinuria in this woman and it is probably due to the severe increase of TG that are used, together with phospholipids, to produce VLDL.

ECOGRAFIA DEI TENDINI ACHILLEI E COMPLICANZE ATEROSCLEROTICHE DELL'IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE

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Obiettivo. È stato ipotizzato che nell'ipercolesterolemia familiare eterozigote (FH) vi sia un parallelismo tra l'entità delle complicanze tendinee (xantomi) e l'entità del danno aterosclerotico, in particolare sembra che i soggetti FH in cui gli XT sono rilevabili all'esame obiettivo abbiano un cardiovascolare ancora più elevato. L'ecografia è in grado di valutare in modo accurato tanto le strutture vascolari quanto quelle tendinee, ed offre perciò un'opportunità di comprendere meglio questi aspetti.
Metodi. Sono stati arruolati 43 soggetti, di cui 11 normocholesterolemici, 10 ipercolesterolemici non affetti da FH e 22 FH, in tutti i soggetti è stata eseguita un'ecografia dei tendini achillei con misura dello spessore tendineo e ricerca di noduli ipo-ecogeni di natura xantomatosa ed un eco-color-doppler delle arterie carotidee e femorali comuni con misura dello spessore medio-intimale e ricerca della presenza di placche.
Risultati. I soggetti FH avevano maggiori valori di spessore medio-intimale (IMT) e maggiore prevalenza di placche aterosclerotiche sia a livello carotideo sia a livello femorale. Solamente nei soggetti FH vi era una correlazione tra i valori di IMT carotideo e spessore dei tendini achillei. I soggetti FH con aterosclerosi prematura avevano valori di IMT carotideo significativamente maggiore, una prevalenza maggiore di xantomi rilevabili clinicamente (40,0 vs 23,5%) ed un maggiore spessore tendineo (11,2 vs 7,4 mm) ma una prevalenza simile di xantomi rilevabili clinicamente.
Conclusioni. Abbiamo dimostrato una correlazione tra lo spessore dei tendini achillei e IMT presente esclusivamente nei soggetti FH. I dati suggeriscono che sia l'entità dello spessore tendineo piuttosto che la semplice presenza di XT ad essere correlata al rischio cardiovascolare.

ECOGRAFIA DEI TENDINI ACHILLEI NELLA DIAGNOSI DI IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE

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Obiettivo. L'ipercolesterolemia familiare eterozigote (FH) è una forma relativamente frequente di ipercolesterolemia primaria, tuttavia molti casi rimangono non diagnosticati, con un conseguente eccesso di mortalità precoce prevenibile. È perciò importante valutare nuove strategie diagnostiche che possano affiancarsi ai tradizionali criteri clinici e biochimici, ed alle tecniche di biologia molecolare. Gli xantomi tendinei sono altamente specifici, pressoché patognomonici di FH, fino ad ora la loro utilità nella diagnosi è stata limitata dalla limitata prevalenza (circa 33% dei soggetti FH adulti). Scopo del nostro studio è stato quindi valutare le caratteristiche diagnostiche dell'ecografia dei tendini achillei nella diagnosi di ipercolesterolemia familiare.
Metodi. Sono stati arruolati 43 soggetti, di cui 11 normocholesterolemici, 10 ipercolesterolemici non affetti da FH e 22 FH, in tutti i soggetti è stata eseguita un'ecografia dei tendini achillei con misura dello spessore tendineo e ricerca di noduli ipo-ecogeni di

natura xantomatosa. È stata valutata la performance diagnostica dell'ecografia vs esame obiettivo nell'identificazione degli xantomi tendinei, ed è stata comparata la performance diagnostica dell'ecografia vs criteri clinici per la diagnosi di FH.
Risultati. I soggetti ipercolesterolemici non-FH avevano uno spessore tendineo sovrapponibile a quello dei soggetti normocolesterolemici, mentre i tendini achillei dei soggetti FH erano significativamente più spessi (5,6±1,2 and 5,6±0,5 vs 8,4±5 mm, p<0,05). L'ecografia tendinea ha mostrato la presenza di alterazioni tendinee patologiche nel 62,5% dei soggetti FH senza xantomi apprezzabili alla palapzione. Sulla base dei risultati dell'ecografia tendinea abbiamo identificato dei criteri diagnostici ecografici sensibili e specifici per la diagnosi di FH (sensibilità 72,7%, specificità 85,7%).
Conclusioni. L'ecografia dei tendini achillei aumenta in modo sostanziale la capacità di identificare gli xantomi tendinei, e facilita così la diagnosi di FH. Alterazioni ecografiche lievi (ma specifiche) sono rilevabili non in una piccola parte, ma piuttosto nella maggioranza dei soggetti FH.

CORRELATION BETWEEN COGNITIVE FUNCTION AND METABOLIC PROFILE IN A COHORT OF ADULT-OLDER PEOPLE: REPORT FROM PANGEA STUDY

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Introduction. Cardiovascular risk factors are established risk factors for vascular dementia however diabetes, hypertension, dislipidemia and central obesity seem to play a role also on Alzheimer's disease development. Aim of this study was to evaluate whether there is an association between metabolic syndrome components (NCEP ATPIII) and cognitive function in a population of adult-older free-living subjects.
Materials and Methods. 299 subjects from general population (mean age 66.28 years [54-81]) were enrolled in the context of PANGeA study. Blood specimens were obtained for biochemical analysis and all subjects underwent a clinical and neuropsychological evaluation (MOntreal Cognitive Assessment). Cardiovascular risk for each participant was calculated based on "Progetto Cuore" algorithm. Statistical analysis was performed with SPSS 22.0 Software.
Results. MOCA score (mean 24.8±2.89 points) positively correlated with HDL cholesterol (R 0.180, P 0.002), and negatively with age (R -0.327, P<0.001), triglycerides (R -0.130, P 0.026), insulinemia (R -0.177, P 0.002), glycemia (R -0.232, P<0.001), HOMA (R -0.214, P<0.001), BMI (R -0.137, P0.018), systolic blood pressure (R -0.161, P 0.005) and waist circumference (R -0.164, P 0.002). The Metabolic Score (the number of metabolic syndrome components) negatively correlated with MOCA score (R -0.164, P 0.005), and also the cardiovascular risk, estimated with "ProgettoCuore" (R -0.361, P<0.001). Finally, at linear model regression analysis, Progetto Cuore risk predicted MOCA score independently of age and schooling (R2 0.315, P<0.001; unstandardized beta coefficient for age: 0.262, for schooling: -0.108, for Progetto Cuore risk estimation: -6.952).
Conclusion. Even in a cohort of non-demented adult-older people, cognitive function is correlated with cardiovascular risk and metabolic syndrome. This condition is well known to be a risk factor for cardiovascular disease and could be associated with a chronic low grade inflammation, a condition associated with both vascular dementia and late onset Alzheimer's disease.

L'ACIDO STEARICO INDUCE LIPOTOSSICITÀ IN VITRO NELLE CELLULE CIRCOLANTI ANGIOGENICHE

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Introduzione. L'insulinoresistenza, soprattutto in condizioni di obesità viscerale, è una condizione caratterizzata da lipotossicità indotta da dagli acidi grassi liberi (FFA). L'esposizione delle isole pancreatiche umane a un'elevata concentrazione di FFA (in particolare di acidi grassi saturi a lunga catena come palmitico e stearico) aumenta notevolmente il tasso di apoptosi β-cellulare, rappresentando uno dei possibili meccanismi che legano l'insulinoresistenza allo sviluppo del diabete. Inoltre, l'aumento in circolo degli FFA, tramite la sintesi intracellulare di ceramide ed attivazione del diacilglicerolo, è in grado di indurre apoptosi anche a livello miocardico, di promuovere la disfunzione endoteliale (ridotta attivazione di eNOS) ed infine di indurre uno stato pro-infiammatorio nei macrofagi, per un'aumentata attivazione del pathway di NF-kB e delle MAPK. Non esistono ad oggi studi riguardanti gli effetti degli FFA sulle cellule circolanti angiogeniche (CACs), che rappresentano il modello di cellule primarie responsabili dei processi di riparazione endoteliale.
Scopo dello Studio. Scopo del nostro studio è pertanto quello di valutare gli effetti degli FFA, in particolare di acido stearico (18:0), sulle CACs ed i possibili meccanismi molecolari coinvolti.
Metodi. Le CACs sono state ottenute da soggetti sani coltivando i linfomonociti su piastre ricoperte di fibronectina in terreno endoteliale per 7 giorni. Acido stearico (SA) complessato alla BSA (bovine serum albumin) è stato aggiunto alle CACs in coltura al fine di valutarne gli effetti:
a) sull'espressione genica di citochine pro-infiammatorie (IL1β, TNFα, IL6, IL8 ed MCP-1 mediante qPCR);
b) sull'attivazione di alcune MAPK (JNK, P38 ed Erk 1/2) e di IkB mediante tecnica western blot;
c) sullo stress del reticolo endoplasmatico (ER) mediante valutazione dell'espressione di CHOP (qPCR);
d) sull'apoptosi (test di attivazione delle caspasi 3 e 7) in esperimenti di dose (da 0 a 800 µM) e tempo (4, 8, 16 e 24 h) risposta.
Risultati. L'aggiunta di SA 100 µM induceva un aumento della risposta pro-infiammatoria nelle CACs (IL-6, IL-8, IL-1β e TNF-α) già dopo 3h di esposizione ed un'attivazione del pathway delle MAPK in particolare di JNK (ma non di P38 ed Erk1/2) e del pathway di NF-kB (dimostrato con l'attivazione e la degradazione di IkB). SA induceva inoltre l'espressione di CHOP a partire dalle 6h di incubazione ad indicare presenza di ER stress, sebbene siano in corso esperimenti per valutare lo specifico pathway molecolare coinvolto. Infine SA induceva attivazione delle caspasi effettrici nelle CACs a partire da 12 h dopo aggiunta di SA e a partire dalla concentrazione 100 µM. L'effetto pro-apoptotico di SA è stato confermato anche dall'espressione dell'Annexina V mediante citofluorimetria a flusso.
Conclusioni. Questi dati, seppure preliminari, dimostrano come la lipotossicità indotta dagli FFA alteri anche i processi di riparazione endoteliale, suggerendo un ulteriore meccanismo alla base della nota associazione tra insulinoresistenza e rischio CV.

NEXT GENERATION SEQUENCING TO IDENTIFY NOVEL GENETIC VARIANTS CAUSATIVE OF MONOGENIC FORMS OF HYPERCHOLESTEROLEMIA: IDENTIFICATION OF A PATIENT

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Objective. Autosomal dominant hypercholesterolemia (ADH) and recessive hypercholesterolemia (ARH) are responsible for very high levels of LDL-C. ADH is caused by mutations in genes including LDLR (LDL receptor, ADH1), APOB (apolipoprotein B, ADH2), and PCSK9 (proprotein convertase subtilisin/kexin type 9, ADH3), while ARH is caused by mutations in LDLRAP1 (low density lipoprotein receptor adaptor protein 1) and possibly CYP7A1 (cytochrome P450, family 7, subfamily A, polypeptide 1).
We investigated a family in which hypercholesterolemia displayed an apparent autosomal dominant pattern of inheritance.
Approach and Results. The proband is a 47-year-old man. His parents are apparently unrelated. His father was reported to be normolipidemic while his mother was reported to have high cholesterol levels (up to 320 mg/dl) and she was on statin treatment. Severe hypercholesterolemia (about 500 mg/dl) and tendon xanthomas was noted when he was 35. At 36 of age he underwent aortic valve substitution. Treatment with statins and ezetimibe was prescribed but plasma total and LDL cholesterol has never reached the target.
To identify the causal mutation in this family we analyzed the candidate genes of recessive and dominant forms of targeted customized sequencing of ADH and ARH genes by Next Generation Sequencing Ion Torrent.
This approach led to the identification of a mutation in LDLRAP1 gene (c.430_431insA; p.His144fs) in homozygosity. The mutation also known as ARH1 and it is one of the most frequent LDLRAP1 mutation in Sardinian population but it is rare outside this geographical area. The identification of this subject carrier of this mutation has prompted us to estimate the allele frequency of ARH1 in two Sicilian free living populations.
Conclusions. We have identified a pathogenic mutation in LDLRAP1 gene in a family with an apparent dominant form of hypercholesterolemia and we evaluated the allele frequency of this mutation in two Sicilian free living populations.

AN APOAV MUTATION (C.427DELC) IDENTIFIED BY EXOME SEQUENCING IS RESPONSIBLE FOR AUTOSOMAL DOMINANT HYPERTRIGLYCERIDEMIA ASSOCIATED WITH PANCREATITS

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Objective. High plasma triglycerides (TG) concentration is a biomarker of a variety of familial and sporadic metabolic disorders. According to a recent proposed simplified definition of the hypertriglyceridemic states, fasting TG plasma levels <2 mmol/l (175 mg/dl) can be considered normal while TG levels between 2 to 10 mmol/l (175 mg/dl - 885 mg/dl) and >10 mmol/l (885 mg/dl) identify subjects with mild to moderate and severe hypertriglyceridemia respectively.
The clinical features of moderate-severe hypertriglyceridemia include mild to severe abdominal pain and recurrent episodes of pancreatitis. We investigated a kindred in which moderate hypertriglyceridemia and pancreatitis displayed an autosomal dominant pattern of inheritance.
Approach and Results. The proband was a 40 year-old male with moderate HTG (418 mg/dl) and he has suffered an episode of pancreatitis. Moderately high plasma levels of triglycerides were observed in 3 more family members. The father of the proband also suffered an episode of acute pancreatitis. A 6 year old girl who is one of the proband's daughters showed TG levels of 198 mg/dl. To identify the causal mutation in this family, we performed exome sequencing in three participants; two with hypertriglyceridemia and previous episodes of pancreatitis and the above mentioned young girl with only hypertriglyceridemia. Approximately 45,000 single nucleotide variants were identified in each sample. After variant filtering, 40 novel shared variants remained. Among these a heterozygous frameshift variant of APOAV gene (c.427 del C; p.R143fs) was identified and confirmed by Sanger sequencing in all 3 studied subjects. This mutation in the heterozygous state has been already described in patients with hypertriglyceridemia.
Conclusions. We used exome sequencing to investigate a kindred with dominant moderate hypertriglyceridemia and pancreatitis. If additional genetic defects besides the APOAV gene mutation are responsible of acute pancreatitis in this family need to be established.

VALUTAZIONE DELL'INDICE DI SARCOPENIA E PULSE WAVE VELOCITY IN SOGGETTI ANZIANI

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Introduzione. La sarcopenia è una sindrome geriatrica caratterizzata da una progressiva perdita di massa muscolare scheletrica e di forza muscolare. La misurazione della velocità dell'onda del

polso (PWV) è un metodo valido e riproducibile per valutare la rigidità arteriosa.
Scopo. Valutare se esiste una correlazione tra i parametri che identificano lo stato di sarcopenia e quelli connessi con la rigidità della parete arteriosa (Pulse Wave Velocity e Augmentation Index).
Materiali e Metodi. Sono stati studiate 72 donne ambulatoriali con un'età compresa tra i 65 e gli 80 anni (età media 71.3±5,2 anni). In tutti i pazienti è stato effettuato un esame densitometrico per valutare il contenuto minerale osseo e la composizione corporea, un prelivo di sangue venoso a digiuno per valutare la calcemia, fosforemia, creatinina, fosfatasi alcalina, 25diidrossi-vitamina D e paratormone, la misurazione della forza prensile della mano con dinamometro elettronico e la Scala di valutazione dello stato mentale. Inoltre tutti i pazienti sono stati sottoposti ad un esame ultrasonografico dei vasi epiaortici con Ecografo Esaote MyLab 60.
Risultati. La sarcopenia è risultata associata ad un peggioramento, anche se non significativo, degli indici di performance fisica (SPPB) e della forza muscolare. I livelli sierici di 25-OH-Vitamina D sono risultati inversamente correlati con la PWV carotidea. La PWV è risultata inversamente correlata sia all'handgrip che all'SPPB. I risultati ottenuti hanno confermato una associazione inversa ma non significativa fra RSMI e PWV.
Conclusioni. La valutazione della sarcopenia basata esclusivamente sulla quantità della massa muscolare appendicolare non sembra costituire un parametro adeguato nel predire sia il grado di rigidità che l'ispessimento medio-intimale delle carotide.

RIDOTTA ATTIVITÀ ENZIMATICA DELLA LIPASI ACIDA LISOSOMIALE IN PAZIENTI NAFLD E NASH

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Introduzione. La lipasi acida lisosomiale (LAL) ha un ruolo fondamentale nel metabolismo lipidico e contribuisce all'omeostasi dei livelli plasmatici delle lipoproteine, prevenendone l'accumulo a livello dei tessuti. La funzione principale della LAL è quella di idrolizzare gli esteri del colesterolo e i trigliceridi. La NAFLD è caratterizzata da accumulo di grasso intra-epatico ed i meccanismi coinvolti nella sua patogenesi non sono ancora chiari. La nostra ipotesi è che una riduzione della LAL possa contribuire ad un accumulo intracellulare di lipidi nei pazienti affetti da NAFLD e NASH.
Materiali e Metodi. Abbiamo studiato l'attività enzimatica della LAL in 240 pazienti NAFLD, di cui 35 NASH, e in 100 soggetti sani (HS). Il dosaggio è stato effettuato su dried blood spot (DBS) preparati depositando 75 µl di sangue intero sulla card. Dopo aver fatto essiccare lo spot overnight si procede con il dosaggio enzimatico mediante metodo fluorimetrico (λex=355nm, λem= 460 nm) utilizzando il substrato artificiale (4-methylumbelliferil palmitate) e il lalistat 2 (inibitore). Il range di riferimento dei soggetti sani (0.95-1.72 nmol/spot/h) è stato ottenuto da 100 adulti privi di danni epatici.
Risultati. L'attività enzimatica della LAL risulta significativamente ridotta nei NAFLD [0.78 nmol/spot/h (0.61-1.01) vs 1.15 nmol/spot/h (0.95-1.72), p<0.001]; una riduzione maggiore è stata osservata nei pazienti con NASH [0.67 nmol/spot/h (0.51-0.77) vs 1.15 nmol/spot/h (0.95-1.72), p<0.001]. I pazienti con attività al di sotto di 1.15 nmol/spot/h mostrano un profilo lipidico caratterizzato da un aumento del colesterolo totale (p<0.05), delle LDL-c (p<0.05) e degli enzimi epatici (ALT, p<0.001; AST, p<0.01; GGT, p<0.01).
Conclusioni. Il nostro studio suggerisce una consistente associazione tra la LAL e NAFLD. Studi più ampi e studi prospettici saranno necessari per approfondire il ruolo della LAL nella patogenesi della NAFLD e per chiarire se il dosaggio dell'attività della LAL possa essere utilizzato come marker non invasivo nei pazienti NAFLD/NASH.

L'ARTROSI COME POSSIBILE FATTORE DI RISCHIO DI MALATTIE CARDIOVASCOLARI NELL'ANZIANO: LO STUDIO PRO.VA.

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Introduzione. Il ruolo dell'artrosi come fattore di rischio cardiovascolare è ancora dibattuto, in particolare nell'anziano. Lo scopo del nostro lavoro è stato investigare se la presenza di artrosi aumentasse il rischio di eventi cardiovascolari in una coorte di persone con più di 65 anni d'età.
Materiali e Metodi. Dei 3.099 soggetti inizialmente inclusi nello studio PRO.V.A. (Progetto Veneto Anziani), 2158 senza malattie cardiovascolari al baseline sono stati seguiti per un periodo di follow-up medio di 4.4 anni. La presenza di artrosi al baseline è stata valutata utilizzando un algoritmo standardizzato che teneva conto di documentazione medica (inclusi accertamenti radiologici), segni/sintomi, ed esame obiettivo delle articolazioni più colpite da artrosi (mano, anca e ginocchio). Come malattie cardiovascolari abbiamo considerato l'insorgenza di coronaropatia, scompenso cardiaco, ictus/TIA, vasculopatia periferica, ospedalizzazione e mortalità per malattie cardiovascolari.
Risultati. Al baseline 1336 partecipanti (=61.9%) soffrivano di artrosi. Le persone con presenza di artrosi avevano una più alta presenza di alcuni fattori di rischio cardiovascolari al baseline (obesità, ipertensione, elevati livelli di LDL e di VES ed una peggior funzione renale) rispetto ai soggetti senza artrosi. Durante il follow-up, 47.8% dei soggetti con artrosi maturava un nuovo evento cardiovascolare vs. il 41.3% di chi non aveva artrosi al baseline. Usando una regressione di Cox aggiustata per potenziali fattori confondenti, la presenza di artrosi aumentava il rischio di eventi cardiovascolari del 22% (HR=1.22, 95%IC: 1.02-1.49, p=0.04). L'associazione tra artrosi ed eventi cardiovascolari risultava più evidente nelle donne, quando l'artrosi colpiva gli arti inferiori e quando più di due articolazioni. Analizzando i singoli outcomes cardiovascolari, la presenza di artrosi aumentava il rischio di coronaropatia, scompenso cardiaco ed ospedalizzazione per malattie cardiovascolari.
Conclusione. L'artrosi predice l'insorgenza di malattie cardiovascolari nell'anziano in particolar modo nelle donne e se gli arti inferiori e più di due articolazioni sono colpite.

EFFICACIA DI UNA MISCELA VEGETALE SOLUBILE A BASSO INDICE GLICEMICO UTILIZZATA COME PASTO IPOCALORICO IN PAZIENTI IN SOVRAPPESO ED OBESI

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Il sovrappeso e, soprattutto, l'obesità rappresentano un importante fattore di rischio cardiovascolare. Scopo dello studio è stato quello di valutare l'efficacia clinica di una miscela vegetale solubile a basso indice glicemico costituita da una polvere (di farina di grano saraceno, di lupini, di riso, di nocciole e di carrube, manna, fibra di avena e vitamina D2 ergocalciferolo, alla dose di due misurini per un totale di 13 g ciascuno per un totale di 26 g, fornente 90 kcal, utilizzata come pasto ipocalorico da miscelare, a seconda del gusto personale, con dell'acqua (un bicchiere) o del latte parzialmente scremato (un bicchiere, circa 60 kcal) o dello yogurt magro (un vasetto, circa 60 kcal) o del succo di frutta (un bicchiere, circa 70 kcal), completato dall'aggiunta di una porzione di frutta per un totale di calorie per pasto oscillanti da un minimo di 90 a un massimo di 250 kcal, in 10 soggetti in sovrappeso (IMC>25-29,9) e 10 obesi (IMC>30) (gruppo d'intervento, trattamento dietetico + miscela vegetale solubile, 2 misurini a pranzo o a cena) versus 10 soggetti in sovrappeso e 10 obesi che hanno continuato il solo trattamento dietetico (gruppo di controllo).
Nel gruppo d'intervento alla settimana 8 si è evidenziato (rispetto al basale), una riduzione statisticamente significativa dell'IMC, della circonferenza addominale, della colesterolemia totale ed LDL, della trigliceridemia, dell'insulinemia e dei valori del rischio cardiovascolare e un aumento statisticamente significativo della colesterolemia-HDL, con riduzione statisticamente significativa del rischio cardiovascolare a 10 anni secondo Framingham (-14,7%). La miscela vegetale solubile, oltre a risultare efficace e a confermare la validità di un approccio basato sul pasto ipocalorico, è risultata anche ben accettata dal punto di vista della palatabilità (con netta preferenza per la miscelazione con latte o yogurt) e ben tollerata.

CHOLESTEROL EFFLUX CAPACITY, HDL-C LEVELS AND CORONARY CALCIUM SCORE AMONG THE VERY ELDERLY SUBJECTS

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Introduction. Cholesterol efflux capacity (CEC) is a functional property of HDL inversely associated to prevalent atherosclerosis and with incident cardiovascular events. The aim of this study was to evaluate serum CEC in very elderly individuals free of cardiovascular events and to correlate it with calcium score as index of subclinical atherosclerosis.
Methods. Healthy individuals aged 80 years or more (n=60), at the 50th higher (68±9.6 mg/dL) and 50th lower (41±9.8 mg/dL) HDL-C levels of the entire healthy cohort (n=208), were selected from The Brazilian Study on Healthy Aging. Serum CEC was measured with radioisotopic techniques by using J774 macrophages treated with a cAMP analogue and exposed to serum of subjects. The Me Multidetector-row cardiac CT for coronary artery calcium score (CACs) was used to detect subclinical atherosclerosis. The presence of not of vulnerable features in the coronary plaques was evaluated by using the Motoyama criteria.
Results. Stratifying according to HDL-C levels, serum CEC did not significantly differ between higher and lower HDL-C levels (12.30±0.92%; compared to 11.81±1.35%). There was no significant difference among the efflux quartiles in terms of CACs and the absence or presence of vulnerable features in the coronary plaques (arterial remodeling, attenuated density or the presence of calcium spots). Serum CEC was slightly but significantly higher in the whole elders group compared to a cohort of 169 subjects of 35-82 years of age (12.10±1.30% compared to 11.32±1.16%; $p<0.001$).
Conclusions. In these healthy very elders individuals serum CEC did not correlate with traditional risk factors such as HDL-C, confirming independence of such functional parameter of HDL-C plasma concentrations. In addition, serum CEC was preserved and not associated to CACs as well as vulnerable features in the coronary plaques. This could be related to the characteristics of participants, that reached old age without manifesting cardiovascular disease.

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