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## A FLEXIBLE METHOD TO SEPARATE IN VITRO EXOMERES AND OTHER EXTRACELLULAR VESICLES TO COMPREHEND THEIR PATHOPHYSIOLOGICAL ROLE. VALIDATION AND POSSIBLE FUNCTIONAL STUDIES

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The poor comprehension of the role of different particles secreted by cells is mainly due to their heterogeneity, coexistence, but also to inadequate separation methods. This situation, together with the recent discovery of exomeres, small (<50 nm) particles with peculiar characteristics, prompted us to propose a new centrifugation-based separation method. Following an algorithm developed by Livshits, we first calculated the theoretical size and then separated five different subfractions (dimensions from >200 nm to <50 nm) of vesicles secreted by a melanoma cell line in vitro. Of these, we studied physical, lipid and protein profiles. Method validation has been accomplished by DLS/Zetasizer, electron- and atomic force microscopy (dimensional analysis), while gas-liquid chromatography, colorimetric assays, LC-MS, CONAN, western blot e MetaCore have been used for lipidomics, proteomics and interactomics. Once we documented the correspondence between theoretical and real dimensions for all the fractions, lipidomics showed a continuous relationship between size reduction, phospholipids' fatty acid saturation and rigidity. The <50 nm fraction, beyond being the stiffest, also presented a different free cholesterol/phospholipid ratio that, together with data emerged from CONAN technique, suggest a different structural diversity, that may be translated into different functions. Proteomics documented the presence of common and unique proteins in each fraction; interestingly, exomeres fraction is characterized by prooncogenic proteins (e.g. MMP-9, TIF-1beta), able to interact in tight networks, thus paving the road of the comprehension of new mechanisms of tumor progression/metastasis. We are currently developing functional studies to ascertain these properties. Altogether:

a) exomeres emerge as the most interesting particles, both biochemically and functionally;
b) the flexibility of our method may allow to deeply study the role of specific particles secreted by different cells in cardiovascular and metabolic pathologies, simply adapting dimensional cut-offs of the centrifugations with the final goal of finding innovative and valid pharmacological treatments.

## METABOLIC ADVERSE EVENTS OF MULTITARGET KINASE INHIBITORS: A SYSTEMATIC REVIEW

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**Introduction.** Multitarget kinase inhibitors are currently used for the treatment of multiple neoplasms, with a significant improvement in survival. The increased life expectancy, such as in the case of thyroid cancer, poses the problem of the development of long-term metabolic side effects. This review aims at investigating the effects of four multitarget kinase inhibitors approved for the treatment of thyroid cancer on glucose and lipid metabolism.
**Materials and Methods.** The literature research lead to the selection of 68 different articles published between January 2012 and December 2022 evaluating the effects of Cabozantinib, Lenvatinib, Sorafenib, and Vandetanib on glucose and lipid metabolism in adult subjects with different malignancies.
**Results.** Elevation of glucose levels (prevalence: 1-17%) was reported in 42 out of 68 studies with different grades of severity, including death. Concerning dyslipidemia, 12 studies reported worsening or new-onset hypercholesterolemia (prevalence: 4-40%), while 19 studies reported different grades of hypertriglyceridemia (prevalence: 1-86%), sometimes leading to life-threatening events.
**Conclusions.** Glucose and lipid levels need to be investigated in future multitarget kinase inhibitors trials since important adverse events may be overlooked if their routine assessment is not performed.

## CAROTID ARTERY DOPPLER ULTRASOUND TO REASSESS CARDIOVASCULAR RISK IN PRIMARY PREVENTION

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**Background.** According to WHO Cardiovascular disease causes nearly 18 million deaths per year (32% of total) of which 85% are caused by Acute Myocardial Infarction and Ischaemic Stroke (atherosclerotic cardiovascular disease acute events). Moreover, Cardiovascular disease causes nearly 40% of early deaths (age <70 years). Thus, clinical activity of Primary Prevention against exposure to modifiable cardiovascular risk factors should be effective and efficient.
**Subjects and Methods.** Two patients with comparable cardiovascular risk category at their clinical presentation were selected from the cohort referring to the Regional Center for Dyslipidaemias of University Hospital of Padua. It was retrospectively speculated if carotid plaque detection with doppler ultrasound (even if not haemodynamically significant plaque) played a role in early high intensity lipid lowering prescription and in keeping up patients in primary prevention.
**Clinical data.** D.A., 56-year-old male, suffered his first myocardial infarction at 52 years. In the next 28 months he had 2 reinfarctions and he was revascularized with a total of 5 percutaneous trans catheter angioplasty and stenting due to a diffused coronary artery disease. At the second reinfarction at 54 years of age, a new coronary angiography documented a no more revascularizable coronary artery disease with a post ischaemic cardiomyopathy. Only 2 years after the index event D.A. was sent to the Regional Center of Dyslipidaemias where it was disclosed that 2 years before the first myocardial infarction, non-haemodynamically significant carotid plaque was detected with doppler ultrasound on top of: former low smoker, dyslipidaemia known from age 43 (total-cholesterol 236 mg/dl, high-density-lipoproteins 41, triglycerides 111, low-density-lipoproteins 173) occasionally treated with low intensity lipid lowering therapy and life style interventions, high arterial blood pressure under aldosterone receptor blockers, impaired fasting glucose, high blood levels of lipoprotein(a) 221 nmol/l detected at the first myocardial infarction and family history of high blood cholesterol and cardiovascular acute events. D.A. ended up reaching his low-density-lipoproteins cholesterol target (<40 mg/dl) on high intensity lipid lowering therapy including mAbs-anti-PCSK9 (evolocumab) only in his tertiary prevention. A.A., 65-year-old female, never suffered myocardial infarction. Negative family history for dyslipidaemia or atherosclerotic cardiovascular disease. Non-smoker. Obese, high arterial blood pressure on drug therapy, type two diabetes mellitus on antidiabetic oral therapy with develop of hypertensive-diabetic nephropathy (microalbuminuria) in her fifth decade of life, hypothyroidism on replacement therapy, dysmetabolic dyslipidaemia known from age 42 with very high single risk factor (total-cholesterol 491 mg/dl lipid lowering therapy naive; after moderate intensity statin total-cholesterol 267 mg/dl, high-density-lipoproteins 51, triglycerides 121, low-density-lipoproteins 192), lipoprotein(a) 120 nmol/l. A.A. at age 42 was sent for the first time at the Regional Center of Dyslipidaemias where non-haemodynamically significant carotid plaque was detected with doppler ultrasound and lipid lowering therapy was tailored to high intensity including mAbs-anti-PCSK9 (evolocumab) as soon as available. A.A. reached her low-density-lipoproteins cholesterol target (<55 mg/dl) in her primary prevention.
**Discussion.** On top of family history of cardiovascular events especially during early age, and personal history of dyslipidaemia and other cardiovascular risk factors, in order to get along with our goal to keep patients at their primary cardiovascular prevention, detection of organ damage as carotid artery atherosclerotic plaque even if non-haemodynamically significant seems to be cost-effective to assess higher risk of fatal and non-fatal cardiovascular events (which could lead to a farer low-density-lipoproteins cholesterol target) and to have a more effective clinical-therapeutical approach with a better impact on long-term quality of life.

## PROGNOSTIC ROLE OF SERUM ALBUMIN LEVELS IN PATIENTS WITH CHRONIC HEART FAILURE

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**Background.** Heart Failure (HF) is a growing health problem, prevalence of 1.5-4% in developed countries. Hypoalbuminemia is common in patients with HF, however there are no data regarding the possible long-term prognostic role of serum albumin (SA) in the younger population with chronic HF, especially in patients without malnutrition.
**Objectives.** The purpose of the present study was to examine the long-term prognostic role of SA levels in predicting major adverse cardiac events (MACE) in middle-aged outpatients with chronic HF, after adjustment for known confounding factors and especially for inflammation, nutritional status, liver and kidney function.
**Methods.** In the present retrospective analysis, 378 subjects with HF were enrolled at the Geriatric Department of "Magna Græcia" University Hospital of Catanzaro, Italy. MACE occurrence (non-fatal ischemic stroke, non-fatal myocardial infarction, cardiac revascularization or coronary bypass surgery and cardiovascular death) was evaluated during a median follow-up of 6.1 years. Total mortality and HF hospitalizations (hHF) were also evaluated.
**Results.** In all population of study 152 patients had a SA value <3.5 g/dL, while the remaining 226 had a SA value ≥3.5 g/dL. In patients with SA ≥3.5 g/dL the observed MACE were 2.1 events/100 patient-year, while in the group with a worse SA levels there were 7.0 events/100 patient-year ($p<0.001$). The multivariate analysis model confirmed that low levels of SA increase the risk of MACE by a factor of 3.1. In addition, the presence of ischaemic heart disease, serum uric acid levels >6.0 mg/dL, chronic kidney disease and a 10-year age rise, increased the risk of MACE in study participants.
**Conclusions.** Patients with chronic HF that exhibits low SA levels showing a higher risk of MACE and hHF.

## ADVANCED GLYCATION END PRODUCTS AS A THERAPEUTIC TARGET IN METABOLIC HEALTH: EVIDENCE FROM A CROSS-SECTIONAL STUDY

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**Background.** Diet quality and energy density play a crucial role in promoting obesity and poor metabolic health. In particular, the typical Western diet, encompassing highly processed, energy-dense foods rich in refined sugars and long-chain saturated fatty acids, is key in the pathogenesis of obesity and its comorbidities. While the effect of nutrients typically consumed as part of the Western diet on metabolic health have been widely investigated, the influence of advanced glycation end products is still to be clarified despite they are also abundant in this dietary pattern (1). Therefore, the aim of this study is to investigate the relationship between advanced glycation end products and metabolic health, with a particular focus on insulin resistance.
**Participants and Methods.** Four Hundred Thirty-Two subjects aged between 55 and 85 years were included in this study and characterized with regard to their anthropometric, nutritional and metabolic profile. Subjects' daily consumption of advanced glycation end products was estimated using food frequency questionnaires and mean of advanced glycation end product content per type of food consumed. The relationship between daily advanced glycation end product intake and anthropometric as well as metabolic parameters was assessed using Spearman test. A simple or stepwise linear regression was performed to evaluate the predictive power of advanced glycation end product intake for cardio-metabolic health parameters. Study participants were stratified into tertiles according to the amount of daily advanced glycation end product consumed. Differences between tertiles were analysed with One Way ANOVA followed by Bonferroni post-hoc test. A p-value ≤0,05 was considered statistically significant.
**Results.** The mean daily advanced glycation end product intake of study participants was 12367,734 kU/day±4389,2381 (standard deviation) in line with previous studies (2, 3). Advanced glycation end product intake positively correlated with (p=0,010) and was also predictive (R^2=0,012; p=0,028) for insulin resistance. In further support of this finding, individuals consuming a diet high in advanced glycation end products had a higher homeostatic model assessment index (p=0,017) compared to those with a lower intake, confirming the association between advanced glycation end product intake and insulin resistance. However, there was no association between advanced glycation end product intake and other predictors of impaired insulin sensitivity (body mass index, fat mass, visceral adiposity index, high-sensitivity C-reactive protein and waist circumference) with the exception of interleukin-18. Stepwise linear regression showed that the predictive power of advanced glycation end product disappeared when considering the aforementioned predictors of insulin resistance. This suggests that advanced glycation end product may influence insulin resistance indirectly, possibly via its modulation of interleukin-18. Indeed, advanced glycation end product consumption positively correlated with and was also a predictor of interleukin-18 circulating levels (R^2=0,009; p=0,046).
**Conclusion.** Dietary advanced glycation end product intake is associated with insulin resistance. However, the impact of these molecules on insulin sensitivity appears to be indirect and potentially mediated via interleukin-18, which is in line with the pro-inflammatory effects of advanced glycation end products and the known role of metabolic inflammation in promoting insulin resistance (4).

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## CV-PREVITAL: A LARGE-SCALE TRIAL ASSESSING THE EFFECTIVENESS OF A DIGITAL INTERVENTION FOR PRIMARY CARDIOVASCULAR PREVENTION

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Cardiovascular (CV) disease continues to be a leading cause of morbidity and mortality worldwide. Despite its significant impact, there is a disproportionate allocation of healthcare budgets towards prevention measures. Therefore, exploration of innovative approaches for effective CV prevention is crucial. Preliminary evidence suggests that digital health interventions could be cost-effective and easily implementable strategies for reducing CV risk. However, robust evidence from large controlled trials is still needed. CV-PREVITAL (ClinicalTrials.gov: NCT05339841) is a multicenter, prospective, randomized, controlled, open-label interventional trial designed to evaluate the effectiveness of a digital intervention in primary CV prevention (BMJ Open. 2023; 13 (7): e072040). The trial involves 14 IRCCS affiliated with the Italian Cardiology Network, along with general practitioners and community pharmacies. The study aims to recruit approximately 80,000 subjects aged ≥45 years without overt CV disease. Participants are randomly assigned to either the control or intervention group. The intervention group receives a personalized prevention program through a smartphone application, targeting lifestyle habits and adherence to pharmacological treatments. The primary endpoint of the trial is the change over a 12-month period in the Moli-sani Risk Score, a new algorithm for measuring the global impact of modifiable CV risk factors (Int J Cardiol. 2023; 389: 131228). Secondary short-term endpoints include the reduction of major CV risk factors. Additionally, the trial includes a long-term (7 years) endpoint to assess the intervention's impact on the incidence of major CV events. Furthermore, CV-PREVITAL incorporates ancillary studies to evaluate the effect of the digital intervention on additional risk biomarkers. Recruitment began in June 2022, and as of September 2023, approximately 20,000 participants have been enrolled. The CV-PREVITAL study will provide valuable data on the efficacy of digital strategies in primary CV prevention, offering insights to inform strategic planning and resource allocation decisions for policy makers. Funding: Italian Ministry of Health (RCR-2019-23669116_001).

## FGF-5 UNIQUELY IDENTIFIES SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN THE POPULATION: RESULTS FROM A PROTEOMICS STUDY

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**Aim.** Heterozygous Familial Hypercholesterolemia (FH) results into lifelong high Low Density Lipoprotein Cholesterol ("LDL-C burden") and premature Atherosclerotic Cardiovascular Diseases (ACVD). An important pharmacological pipeline dedicated to FH has been developed but its efficacy is still sub-optimal, due to a substantial probability to misdiagnose FH as subjects with similarly elevated LDL-C but not carriers of FH-associated mutations ("non-FH"). We sought novel plasma biomarkers for FH that might help to surmount these diagnostic shortcomings.

**Methods.** At Milan, we compared the Normalized Plasmatic eXpression (NPX, Olink) of 264 proteins in 133 FH (8% on statins) with that of 586 normocholesterolemic subjects ("controls") and that of 55 non-FH (LDL-C 190-220 mg/dL, not on statins). We harnessed machine learning to look for the most representative proteins of FH. We checked for validation in:

1) an additional group of 76 FH from the Lipid Clinic of Palermo and
2) in two Dutch general populations (10 non-FH vs 657 controls from the Athero-Express and 33 non-FH vs 837 controls from the Second Manifestations of ARTerial disease (SMART) study).

**Results.** An increased plasmatic expression of Fibroblast Growth Factor 5 (FGF-5) potently differentiated the FH subjects from the controls (Area Under the Curve (AUC)=0.995 (0.985-1.000); P<0.0001) and from the non-FH (AUC= 1.000 (0.914-1.000); P<0.001). The robust association between FGF-5 and FH was confirmed in the FH from Palermo. Vice versa, FGF-5 was not able to distinguish non-FH from controls, a finding that was confirmed in Athero-Express and SMART.We finally confirmed NPX data with ELISA-based quantification.

**Conclusion.** FGF-5 appears a clear-cut biomarker unique for FH. The LDL-C burden can be responsible but, as we preliminary ruled-out a physical interaction between FGF-5 and LDL receptor, proteomics might inform on pathways independent from those targeted by the canonical pharmacological tools.

## SPIKE/ESTROGEN RECEPTOR-α INTERACTION: AN UNESPECTED WAY TO UNDERSTAND THE HYPERACTOVATION OF COAGULATION CASCADE BY SARS-CoV-2

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**Background.** Coagulopathy and thrombi formation has been observed in several organs in patients with COVID-19, and in a minority of subjects receiving the SARS-CoV-2 vaccine. The correlation between SARS-CoV-2 infection and hyperactivation of coagulation cascade has been established. Several mechanisms have been proposed to explain this relationship. However, the direct effects of SARS-CoV-2 on the transcriptional machinery controlling these pathways are not completely understood. Recently, we suggested a novel transcriptional function of the SARS-CoV-2 Spike (S) viral protein involving a direct protein-protein interaction with the human Estrogen Receptor-α (ERα). Given the known implications of ERα in the control of key regulators of the coagulation cascade in endothelial cells, we hypothesized that S-protein might increase the coagulation response in vitro and in vivo via ERα.

**Methods.** S-proteins were electroporated in the abductor muscles in mice, and recalcification time and the levels of D-Dimer, von Willebrand Factor (vWF) and Tissue Factor (TF) were measured in plasma. Human endothelial cells (Ea.Hy926 and HULEC-5a), inhibitors of the ERα (Raloxifene, Fulvestrant), S-proteins, and the natural ERα agonist, the 17-β-Estradiol were used for in vitro studies. TF expression was investigated in cell lysed and in cell supernatant by RT-qPCR and by ELISA, respectively, and procoagulant activity (PCA) was assessed in treated cells.

**Results.** We showed that S-protein promoted the expression of TF and the overall pro-coagulation activity in a endothelial cells and confirmed this finding by overexpressing S-protein by gene transfer in mice. Then, due to the knowledge based on the EXSCALATE supercomputing platform, we designed and tested two-point mutations in the S-sequence that abolished the pro-coagulation function of Spike in vitro and in vivo, without compromising its immunogenicity.

**Conclusions.** Our findings suggest a new non-infective pathologic action of the Spike-protein at the vascular level promoting the endothelial pro-coagulation activity by a crosstalk with the ERα.

## EFFECTS OF TRADITIONAL CIGARETTE AND TOBACCO HEATING PRODUCT ON VASCULAR SMOOTH MUSCLE CELL SENESCENCE

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Cellular senescence, a process that imposes permanent proliferative arrest, senescence-associated secretory phenotype (SASP) and high level of reactive oxygen species (ROS) on cells, has emerged as a potentially important contributor to aging and age-related diseases. Senescent vascular smooth muscle cells (VSMCs) are present in atherosclerotic plaques and contribute to their instability. Traditional cigarette (TC) smoke is a known exacerbator of age-related. To reduce the harm associated with TC, alternative next-generation tobacco products (NGTPs), such as tobacco heating products (THP), have been developed. We studied the effects of aqueous extracts (AEs) from TC and THP on VSMC senescence compared to doxorubicin-induced senescence (DIS) cells. VSMCs passaged 5 to 7 times (young cells) were incubated for 48 h with 10% TC and THP AEs or doxorubicin 100nM. We measured SA-β-gal activity (senescence marker), senescence associated genes and proteins expression, cell proliferation, cell cycle, ROS production and nuclear changes. TC increased SA-β-gal positive cells (more than 50%), slowed cell proliferation down to a permanent growth arrest and down-regulated proliferation cell nuclear antigen (PCNA) expression, similar to what we observed in the DIS VSMCs. Instead, THP did not affect cell proliferation nor PCNA expression. Despite expression of cell cycle inhibitors was upregulate in both TC and THP, only TC arrested cells at G2/M phase. Moreover, only TC significantly induced SASP expression and increased the production of both mitochondrial and intracellular ROS. Once again, THP did not show any effect. Finally, TC, and not THP, increased the number of large and regular nuclei, a typical senescent nuclei feature.
In conclusion, TC was able to induce premature senescence in VSMCs at a level similar to DIS. THP lacked most of the TC and doxorubicin-related functional, structural, and molecular effects on VSMCs senescence. The real long-term health effects of these NGTPs will have to be further assessed.

## LDL-CHOLESTEROL TARGET LEVELS ACHIEVEMENT IN HIGH-RISK PATIENTS: AN (UN)EXPECTED GENDER BIAS

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**Background and Aims.** Lowering low-density lipoprotein cholesterol (LDL-C) is the cornerstone of cardiovascular disease prevention. Collection of epidemiological data is crucial for monitoring healthcare appropriateness. This analysis aimed to evaluate the proportion of high-risk patients who achieved guidelines recommended LDL-C goal, and explore the predictors of therapeutic failure, with a focus on the role of gender.
**Methods and Results.** Health administrative and laboratory data from seven Local Health Districts in Tuscany were collected for residents aged ≥45 years with a history of major adverse cardiac or cerebrovascular event (MACCE) and/or type 2 diabetes mellitus (T2DM) from January 1, 2019, to January 1, 2021. The study aimed to assess the number of patients with optimal levels of LDL-C (<55 mg/dl for patients with MACCE and <70 mg/dl for patients with T2DM without MACCE). A cohort of 174 200 individuals (55% males) was analyzed and it was found that 11.6% of them achieved the target LDL-C levels. Female gender was identified as an independent predictor of LDL-C target underattainment in patients with MACCE with or without T2DM, after adjusting for age, cardiovascular risk factors, comorbidities, and district area (adjusted-IRR 0.58, SD 0.01; p <0.001). This result was consistent in subjects without lipid-lowering therapies (adjusted-IRR 0.56, SD 0.01; p <0.001).
**Conclusion.** In an unselected cohort of high-risk individuals, females have a significantly lower probability of reaching LDL-C recommended targets. These results emphasize the need for action to implement education for clinicians and patients and to establish clinical care pathways for high-risk patients, with a special focus on women.

## ANGPTL3 AND PCSK9 DETERMINE A SHIFT IN THE ACTIVATED METABOLIC PATHWAYS IN A HEPG2 CELLULAR MODEL

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**Background and Aim.** ANGPTL3 and PCSK9 are known regulators of lipoprotein metabolism. Patients affected by familial hypobetalipoproteinemia type 2, due to homozygous loss of function of the ANGPTL3 gene, show reduced levels of circulating PCSK9 indicating a possible coordinate regulation of these two proteins. Our research aims to establish whether the two proteins are able to determine a metabolic shift in a liver cellular model.
**Methods.** HepG2 cells were cultured in fasting (low-nutrient medium) or feeding conditions (high-nutrient medium). We generated ANGPTL3 and PCSK9 over-expressing (OE) cells and used a proteome profiler to study intracellular activated pathways. Based on the activated transcription factors found in the proteome profiler we performed chromatin immunoprecipitation (ChIP) and we explored the specific activation of targets of lipolysis and lipogenesis using real-time quantitative PCR (qPCR).
**Results.** At the proteome profiler, ANGPTL3 OE cells show an activation of the IGF-1 receptor, PLC-gamma, and STAT3. PCSK9 OE cells show the activation of different TRK receptors and the intracellular activation of STAT1. Double OE cells re-establish intracellular signaling similar to CTRL cells. The ChIP showed a STAT1-mediated expression of genes involved in mitochondrial beta-oxidation when PCSK9 was overexpressed and increased STAT3-mediated expression of genes involved in cholesterol metabolism when ANGPTL3 was overexpressed.
**Conclusions.** ANGPTL3 activates the IGF1R-PLC-γ-STAT3 axis associated with increased cholesterol synthesis and ApoB maturation. Conversely, PCSK9 activates the STAT1 pathway associated with increased mitochondrial beta-oxidation.

## IMPACT OF IMMUNE SYSTEM HUMANIZATION ON ATHEROSCLEROSIS IN DYSLIPIDEMIC RAG2-KO/IL2RG-KO/ CD47-KO/LDL-R KO MICE

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**Background and Aim.** Given the key role of the immune response during atherosclerosis and the therapeutic interest of biologics targeting human immune cells, the need of experimental models to translate molecular mechanisms and to test therapeutic approaches for atherosclerosis is continuously increasing. Here we describe the characteristics of an innovative immunodeficient mouse humanized with human hematopoietic cells on an atheroprone.
**Background. Methods.** LDLR-KO mice were crossed with the immunodeficient C57BL/6J strain Rag2-KO/IL2rg-KO/CD47-KO (TKO, IMSR_JAX:025730) to generate an immunocompromised dyslipidemic mouse recipient TKO-LDLR KO of human hematopoietic stem cells (hCD34+).
**Results.** TKO-LDLR KO were first characterized for their immune and metabolic profile. TKO mice are deficient in mature lymphocytes and NK cells and this profile was conserved in TKO-LDLR KO mice. Under high cholesterol diet, TKO-LDLR KO present monocytosis with increased levels of Ly6Chi monocytes compared to TKO-LDLR KO at standard diet, develop marked dyslipidemia, steatosis and atherosclerosis. This profile confirms the suitability of TKO-LDLR KO mice for atherosclerosis studies. Next we tested the impact of immune system humanization on atherosclerosis. TKO-LDLR KO pups received a low-dose irradiation (150-200 cGy) and thereafter 1,5-2 x 10^5 hCD34+ were injected with in the liver. Engraftment of human leukocytes (hCD45+) was evaluated after two months by flow cytometry analysis from tail blood. This approach allows to reconstitute between 10-30% of hCD45+, mainly B and T cells. The humanization with hCD34+ cells affected atherosclerosis development as compared to non-humanized TKO-LDLR KO mice.
**Conclusion.** We have generated and characterized for the first time a humanized dyslipidemic TKO-LDLR KO mice. This mouse model presents human B and T cells and represent and important toll to investigate the impact of biologics targeted toward human targets in the context of atherosclerosis.

## THE IMPACT OF SOLUTE CARRIER ORGANIC ANION TRANSPORTER 1B1 (SLCO1B1) RS4149056 VARIANT ON LOW-DENSITY LIPOPROTEIN CHOLESTEROL TARGET ACHIEVEMENT AFTER LIPID LOWERING THERAPY OPTIMIZATION IN MEN AND WOMEN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Aim.** This retrospective observational study evaluated the impact of the genetic variant SLCO1B1 rs4149056 on LDL-C target achievement after lipid lowering therapy optimization in men and women with FH.
**Methods.** The study involved 412 FH subjects with probable or defined clinical FH diagnoses, who underwent genetic analysis between June 2016 and September 2022 at the University Hospital of Catania's referral Lipid Center. Biochemical analyses were conducted at baseline and during the last follow-up (January to June 2023) after optimizing lipid-lowering therapy, following the 2019 ESC/EAS Guidelines on Dyslipidemias.
**Results.** The SLCO1B1 rs4149056 variant was present in 24.5% of FH subjects, with no significant gender-based variation. At baseline, both men and women had low percentages of subjects meeting LDL-C targets. Moreover, fewer women were on lipid-lowering therapy and high-intensity statins compared to men. After therapy optimization, only 33.0% achieved target LDL-C levels, with 70.3% of women not on high-intensity statins. Moreover, women reported higher rates of statin-associated side effects (SASE) and myalgia than men. Stratifying by gender and genetic variant, significant decrease trends of FH subjects on high-intensity statin as well as on LDL-C target were found from M/SLCO1B1- group to F/SLCO1B1+ group, while the proportions of subjects with SASE fear as well as reported myalgia increased from M/SLCO1B1- group to F/SLCO1B1+ group. Logistic regression analysis showed that M/SCLO1B1+, F/SCLO1B1- and F/SCLO1B1+ groups were inversely associated to LDL-C target achievement and F/SCLO1B1+ group reported the strongest association.
**Conclusions.** Long term adherence of high-intensity statin was lower in FH women than men; thus, a low prevalence of FH women achieved the recommended LDL-C target. FH women with SLCO1B1 rs4149056 variant presented the lowest proportions of subjects on high-intensity statin as well as on LDL-C target; finally, FH women with SLCO1B1 rs4149056 variant reported the strongest inverse association with LDL-C target achievement.

## COMPARISON OF TWO POLYGENIC RISK SCORES TO IDENTIFY NON-MONOGENIC PRIMARY HYPOCHOLESTEROLEMIAS IN A LARGE COHORT OF ITALIAN HYPOCHOLESTEROLEMIC SUBJECTS

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**Introduction.** Primary Hypobetalipoproteinemias (HBL) are a group of dominant and recessive monogenic genetic disorders caused by mutations in APOB, PCSK9, ANGPTL3, MTTP, Sar1b genes and characterized by plasma levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5$^{th}$ percentile of the distribution in a given population. Mutations in the candidate genes account only for a small proportion of subjects with HBL suggesting a role for a polygenic contribution to the low cholesterol phenotype.
**Material and Methods.** To explore the complex genetic architecture of HBL we compared two polygenic risk scores in order to assess the role of the polygenic burden and the differences in the clinical phenotype between monogenic and polygenic HBL; we studied a cohort of 170 subjects with primary HBL referred over a 25-year period to 2 Italian reference centers have been studied. The genetic analyses have been based on: Sanger sequencing, in-house NGS customized panel and two scores, PRS1 and PRS2 for the polygenic burden.
**Results and Conclusion.** Sixty 60 (35%) and 63 (37%) subjects had a monogenic and polygenic HBL respectively. LDL-C plasma levels were significantly lower in monogenic HBL (30.87±3.12 mg/dl) compared with the non-monogenic HBL (42.80±2.18 mg/dl) ($p<0.002$) with no differences in the percentage of fatty liver. Only PRS1 is effective in detecting polygenic HBL while PRS2 does not improve the polygenic diagnosis.

## ATTITUDE IN CONSUMING SPICY FOODS IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Aim.** Familial Hypercholesterolemia (FH), probably the major genetic risk factor in the development of cardiovascular diseases in adult age, is of mounting interest in pediatric age too, since early intervention may significantly reduce mortality and morbility. Many nutraceuticals and phytochemical supplements serve as a promising complementary therapy. Among them, spicy foods have attracted special attention. Capsaicin is the main active ingredient in chili peppers and spicy food. Plasma lipid-lowering activity of garlic, ginger, and turmeric have been well-studied in both humans and animals, while effects of chili and black peppers on blood cholesterol is under investigation. However, taste of spice is not always appreciated by people in general, and children in particular.
**Material and Methods.** During the period 1 NOV 2022 - 31 JAN 2023 we interviewed all children referring to our Pediatric Ambulatory of Familial Hypercholesterolemia of Bambino Gesù Children's Hospital in Rome, Italy. We collected data on age, sex, region of residency and attitude/frequency to assume spicy food (chili or other spicy).
**Results.** On a total of 87 children interviewed, 20% (18/87 children, 8 F/10 M, range 8-15 yrs, median age 11,4 yrs) declared to regularly assume spicy foods. While all eat chili, at least 10/18 (55%) eat other various spicy foods too. More than 70% (13/18) declared to eat spicy foods more than 4 times per week. Interestingly, just 3/18 lived in Italian regions were spicy foods play a key role in traditional cuisine ( i.e. Calabria), while other children came from a variety or geographic areas, suggesting that comsumption of spicy foods is an attitude that is growing worldwide.
**Conclusions.** According to our results, spicy foods is appreciated by a not insignificant percentage of children. More studies are needed to define their role as complementary therapy in FH.

## DIETARY LIPIDS AFFECT MORE THAN GENOTYPE HEPATIC AND INTESTINAL MIRNOME: A STUDY IN MOUSE MODELS WITH DIFFERENT LIPID PROFILES

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**Aim.** To generate an atlas of miRNA expression levels in liver and small intestine of mice characterized by a different lipoprotein metabolism.
**Materials and Methods.** C57BL/6J, Ldlr-KO and Pcsk9-KO male mice (n=3/group) were fed a Chow diet or a Western diet for 16 weeks. Plasma lipids as well as miRNomes of liver, duodenum, jejunum and ileum were assessed.
**Results.** Cholesterolemia and triglyceridemia were higher in Ldlr-KO than in the other genotypes, under both Chow and Western. On Chow diet, C57BL/6J were characterized by higher cholesterolemia and triglyceridemia than Pcsk9-KO, but no differences were observed between the two genotypes on Western diet. In each genotype, Western diet-fed mice had higher cholesterolemia and triglyceridemia than Chow-fed mice. In the liver, the expression of 11 miRNAs varied significantly in all genotypes when comparing Chow and Western diets (miR-7a-5p, miR-381-3p, miR-96-5p, miR-136-5p, miR-434-3p, miR-409-3p, miR-300-3p, miR-127-3p, miR-431-5p, miR-582-5p, miR-34a-5p). Ninety-two out of their 7944 predicted target genes were related to lipid/lipoprotein metabolism. Several among these miRNAs were capable of synergistic action against their targets: 41 lipid-related genes were targeted by at least 3 diet-modulated miRNAs. Of note, Zbtb20, a gene involved in hepatic de novo lipogenesis, appeared to be simultaneously modulated by 9 miRNAs. The intestinal segments responded differently to the dietary treatments and did not share any differentially expressed miRNA in duodenum and jejunum. In the ileum, the expression of 4 miRNAs varied significantly in all genotypes when comparing Chow and Western diets (miR-29a-5p, miR-223-3p, miR-98-3p, miR-467d-5p). Among the 583 predicted target genes, 22 transcripts were related to intestinal lipid/lipoprotein metabolism and were all modulated by mmu-miR-98-3p.
**Conclusions.** Our study demonstrates that:
1) diet- and genotype-determined dyslipidemic conditions impact on the miRNome in an organ-specific manner;
2) dietary lipids represent a much more effective driver than genotype in altering miRNA expression.

# CYCLIC FASTING BOLSTERS CHOLESTEROL BIOSYNTHESIS INHIBITORS' ANTICANCER ACTIVITY

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**Introduction.** The development of new cancer treatments is a long and costly process with high attrition rates before reaching Phase II trials. Identifying oncological applications for drugs that are already approved for other medical indications is considered to be a possible solution to overcome this issue (1). Incorporating dietary interventions such us fasting or modified fasting into cancer therapy holds promise as means to potentiate the efficacy of existing anticancer therapies (2, 3) (chemotherapeutics, tyrosine kinase inhibitors, endocrine therapy and immune checkpoint inhibitors) and to enhance the antitumor properties of drugs that are normally used for non-oncological indications (e.g., the antidiabetic drug, metformin, and vitamin C). For this reason, there is strong rational to hypothesize that dietary interventions will help drug repurposing in oncology, unleashing anticancer effects of already approved drugs. Alterations of cholesterol (CE) homeostasis can promote tumor development and progression (4). Indeed, the upregulation of the CE synthesis pathway plays a crucial role in maintaining the growth and the metastatic spread in pancreatic ductal adenocarcinoma models (PDAC). Moreover, several enzymes involved in this pathway are deregulated in cancer cells. In addition, CE accumulates in membrane domains to form liquid-ordered domains known as lipid rafts, which, regulate numerous signaling pathways controlling cell proliferation. Thus, targeting de novo CE biosynthesis pathway represents an attractive therapeutic strategy.
**Materials and Methods.** In vitro, fasting condition were mimic with low-glucose (0.5 g/L) DMEM medium with 1% FBS. Mice subcutaneously injected with human PDAC or colorectal cancer (CRC) cells underwent weekly cycles of 48 hours fasting and tumor size and mice weight were monitored daily. Gene and protein expression and cholesterol levels were determined on cells and tumor masses.
**Results.** Screening over 800 approved drugs in PDAC cells, we identified three fungicidal agents, clotrimazole, miconazole and oxiconazole, to became strongly cytotoxic against cancer cells when combined with fasting conditions. Since azoles inhibit 14alpha-demethylase, a key enzyme for CE biosynthesis, we hypothesized that fasting and azoles would cooperate by blunting CE production in cancer cells. Consistently, we found that also simvastatin and terbinafine, inhibitors of other two key enzyme in CE production, had their antitumor effects strongly enhanced by fasting on several cancer cell lines and on tumor organoids from patients with CRC. Thus, fasting conditions cooperated with these agents to reduce intracellular CE by downregulating genes involved in its production and increasing CE efflux. To further investigate the role of fasting and CE biosynthesis inhibitors against cancer, we injected human PDAC and CRC cells in mice and we saw that fasting enhanced clotrimazole and terbinafine antitumor effects, blunting the CE levels in the masses. In addition, combined fasting and terbinafine lowered circulating low-density lipoprotein cholesterol (LDL) and triglycerides, whereas increased high-density lipoprotein cholesterol as compared to terbinafine alone. We performed the same experiments adding intraperitoneal injection of human plasma LDL and we found that the addback was sufficient to revert the fasting-induced enhancement of drugs anticancer activity and abrogated the reduction of intratumor CE content. Consistent with CE being an essential constituent of membrane lipid rafts, that harbor the growth-promoting PI3K/AKT signaling cascade, combined terbinafine or clotrimazole and fasting strongly downregulated phosphorylated AKT and its downstream target. In addition, combined therapy reduced mitochondrial oxidative phosphorylation and energy status in gastrointestinal tumor xenografts, whereas CE restoration abolished this effect.
**Conclusions.** Taken together, these findings support the rationale for conducting clinical studies to assess the safety, feasibility and efficacy of combining periodic cycles of fasting with inhibitors of CE biosynthesis in cancer patients.

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## IMPACT OF CARDIOVASCULAR RISK FACTORS ON ARTERIAL VASCULAR REMODELING IN A COHORT OF PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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**Background.** The immune system plays a central role in the atherosclerotic process. Common Variable Immunodeficiency (CVID) is a rare immunological disease characterized by a deficiency in antibody production due to the altered functionality of B lymphocytes. The actual impact of cardiovascular (CV) risk factors on the progression of arterial organ damage in these patients is uncertain.
**Objective.** To evaluate the impact of classical CV and immunological risk factors on the early arterial damage in CVID patients.
**Materials and Methods.** We performed an observational study on 59 patients affected by CVID and followed at the Regional Center for Rare Immunological Diseases at the Treviso Hospital. For each patient we collected anamnestic information, anthropometric data, blood pressure values, biohumoral parameters and immunological characteristics. Vascular organ damage was estimated by determining arterial stiffness (PWV) and the intimal media thickness of the common carotid artery (IMT).
**Results.** The median age of the population was 54 years (RIQ 42.5-61.5) with a prevalence of female (64.4%). Only one patient had a history of atherosclerotic cardiovascular events. To determine the impact of CV risk factors on vascular damage, patients were stratified based on the presence or absence of: overweight (BMI >25 kg/m$^2$), arterial hypertension (BP >140/90 mmHg), dyslipidemia (LDL >115 mg/dl), metabolic syndrome and immunological phenotype according to Chapel classification (infection only vs complicated). Patients with hypertension (p=0.003), overweight (p=0.001), dyslipidemia (p=0.04) and metabolic syndrome (p=0.01) showed a significant increase in PWV values compared to CVID patients without these characteristics. On the contrary, no difference was observed between the different subgroups in term of carotid IMT values. Stratification according to the Chapel classification revealed no differences in the two clinical phenotype for both PWV and carotid IMT.
**Conclusions.** In CVID patients, CV risk factors determine an increase in arterial stiffness parameters in the absence of significant differences in early atherosclerotic damage. Further studies are necessary to establish whether dysregulation of the immune system may be associated with a reduction in the progression of atherosclerosis.

## ANTI-INFLAMMATORY AND ANTI-ATHEROSCLEROTIC PROPERTIES OF INTESTINAL METABOLITES OF HIGH-AMYLOSE WHEAT PHENOLIC EXTRACT

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Dietary polyphenols are recognized as protective compounds against atherosclerosis by alleviating vascular inflammation and improving endothelial function. Whole wheat is a rich source of phenolic compounds with athero-protective properties. However, the effects of intestinal metabolites of wheat phenolic compounds are poorly understood. Newly developed high-amylose durum wheat cv. Svevo (Svevo-HA), with a high content of resistant starch and phenolic acids, is gaining interest as a healthy ingredient for functional food. The present study aimed to investigate the effects of the intestinal metabolites of high-amylose wheat phenolic extract (WPE) on endothelial inflammation and endothelium-leukocyte adhesion, a crucial step in atherosclerosis. We established a transwell epithelial-endothelial co-culture system with Caco-2 and HMEC-1 cells mimicking the intestinal and vascular layer, respectively. Caco-2 cells were grown on semipermeable filters until fully differentiated into an enterocyte-like phenotype, and then they were moved to plates containing HMEC-1 in the lower compartment. WPE (1-10 μg/mL gallic acid equivalents) was added on the apical compartment for 2 h. Afterwards, TNF (10 ng/mL) was applied on the basolateral compartment for 16 h to simulate the inflammatory milieu. Endothelium-leukocyte adhesion, the expression of endothelial inflammatory mediators, intracellular ROS levels and NF-κB activation were evaluated by multiple assays. The results showed that trans-epithelial WPE suppressed, dose-dependently, the TNF-induced expression and release of interleukin-6 and monocyte chemoattractant protein-1, as well as, reduced the expression of endothelial adhesion molecules, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, and subsequent endothelium-leukocyte adhesion. The anti-inflammatory effects of WPE intestinal metabolites were mediated by reduction of NF-κB activity and intracellular ROS. In conclusion, these results suggest Svevo-HA as an excellent functional food ingredient rich in phenolic compounds with multiple anti-inflammatory and anti-atherosclerotic properties, which could help counteract or prevent inflammatory vascular diseases. (Funding: PRIMA Section 1, 2020 Agrofood Value Chain IA, MEDWHEALTH, grant n. 2034).

## RELATIONSHIP BETWEEN GUT PERMEABILITY AND PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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**Background and Aims.** Gut dysbiosis is a major determinant of low-grade endotoxemia via dysfunction of the intestinal barrier scaffold, which is a prerequisite for Lipopolysaccharide (LPS) translocation into the systemic circulation. Endotoxaemia is associated with atherosclerotic burden and its clinical sequelae. Moreover, current data show that LPS is cleared from the circulation via low-density lipoprotein receptors (LDLR) on hepatocytes, which are downregulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein directly associated with circulating LDL cholesterol (LDL-C) level and in the onset of hypercholesterolemia. The aim was to analyze the relationship between PCSK9, gut permeability and endotoxemia after the treatment with PCSK9 inhibitors (PCSK9i).
**Materials and Methods.** We performed a before-after study including 40 patients with heterozygous familial hypercholesterolemia (HeFH) on treatment with maximum tolerated statin dose ± ezetimibe before and after six months of PCSK9i therapy. We analysed plasma PCSK9 levels, intestinal permeability marker such as zonulin, endotoxemia markers such as LPS and oxidized-LDL (ox-LDL) that play a central role in the atherosclerotic process, by enzyme-linked immunosorbent assay (ELISA).
**Results.** We observed a significant decline in LDL-C, zonulin, LPS, and ox-LDL levels after six months of PCSK9i compared to baseline. Furthermore, linear regression analysis showed that LPS levels were associated with LDL-C and zonulin reduction, suggesting a relationship between gut permeability and PCSK9 levels.
**Conclusions.** This study provides evidence that PCSK9i could improve intestinal permeability by reducing circulating endotoxemia as well as oxLDL production. This is a novel pathway that could counteract the atherosclerotic process in HeFH patients.

## DIAGNOSIS AND LONG-TERM FOLLOW-UP OF CHILDREN AND ADOLESCENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA AT A PEDIATRIC LIPID CENTRE

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**Introduction and Aim.** Several intervention studies have evaluated dietary and pharmacological treatment in children and adolescents with familial hypercholesterolemia (FH); however, scientific works documenting follow-up data are rather scarce. The aim of the study is to assess the time of diagnosis and the long-term follow-up of FH children and adolescents at a pediatric Lipid Centre.
**Patients and Methods.** 96 FH children, aged 2-13 years (mean: 7 years 3 months; M:50, F:46) were retrospectively analysed. The cohort was divided into 3 age groups at diagnosis: 1-5y; 6-10y, 11-13y. We evaluated: treatment, mean reduction in lipid values, age of drug therapy initiation, at times (years): baseline (T0), T1, T4, T7 from the diagnosis. The population was also assessed by family history for cardiovascular disease (CVD+/-) and by the presence of pathogenic mutation on LDL-R (MUT+/-).
**Results.** After 7 years from the time of diagnosis (T7) the percentage of subjects on drug therapy was: 64.7% (1-5 y); 29.4% (6-10 y); 18.2% (11-13 y); overall cohort LDL-C reduction (mg/dl): -35.3 ($p<0.001$), in the subgroups: LDL-C: -54.4 ($p<0.001$); -23.8 ($p<0.001$); -4.8 (p: 0.653), respectively. Considering family history (CVD+/-) at T7: 54.2% vs 27.1% on drug therapy; age at diagnosis: 6y 4m vs 7y 3m (p: 0.909); age at drug therapy initiation: 11y 2m vs 12y 7m (p: 0.039). Assessing in relation to genetic analysis (MUT+/-): 67.7% vs: 27.7% on drug therapy at T7; age at diagnosis: 5y 8m vs 7y 4m (p: 0.004); age at drug therapy initiation: 10y 7m vs 10y 8m (p: 0.869).
**Conclusions.** In the 7-year follow-up of FH children, we documented an improvement of the lipid profile after the treatment. We also found that MUT+ children, were diagnosed at an earlier age and CVD+ children started drug therapy at an earlier age than CVD- subjects. Treatment of familial hypercholesterolemia is feasible from pediatric age and it is effective and well tolerated.

# CAN GOOD PRESSURE CONTROL IN THE OVERWEIGHT POPULATION BE SUFFICIENT?

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**Background.** Among cardiovascular risk factors in clinical practice, particular attention is paid to obesity, but often that leads to underestimating the population of overweight patients. In this sense, echocardiographic examination could play a role in regulating therapeutic aggression in hypertensive patients, even with a good blood pressure control, in the absence of overt organ damage.
**Aim.** Evaluate whether overweight subjects have early echocardiographic signs of left ventricular remodeling compared to normal-weight subjects with equal pressure control and in the absence of comorbidity.
**Methods.** We considered patients treated in our hypertension center in 2021 who underwent an echocardiography evaluation at our dedicated clinic. Inclusion criteria were: absence of comorbidity and outpatient blood pressure monitoring that showed good blood pressure control during the 24 hours (<130/80 mmHg), for a total of 29 patients.
We then further layered them for day (<135/85 mmHg) and night (<120/70 mmHg) pressure control. The patients were then divided according to BMI in normal weight (18.5-24.9) and overweight (24.9-29.9) and were evaluated their respective echocardiographic parameters.
**Results.** The study found that the overweight population (n 16) vs normal weight population (n 13) had significantly higher values of interventricular septal thickness (IVST), left ventricular posterior wall (LVPW), and left atrial diameter (LAD). This finding was confirmed even after dividing the patients according to day and night pressure control.
**Conclusion.** In our work, we highlighted how overweight patients, with equal pressure control, present early signs of cardiac organ damage induced by hypertension, specifically identified in the parameters of IVST, LVPW, and LAD. Despite the limitations of the small number, this study highlights the need to pay more attention to this population, even in the absence of other comorbidities, whose cardiovascular risk is often underestimated.

**Table 1** - Population characteristics. BMI = Body mass index; SBP = systolic blood pressure DBP = diastolic pressure (DBP) blood.

| Population characteristics | | | |
|---|---|---|---|
| | Normal weight | Overweight | p-value |
| Mean age (years) | 64 ± 9,17 | 67 ± 10,31 | 0,751 |
| Women (%) | 46,15 | 37,5 | 0,878 |
| BMI (kg/m2) | 23,02 ± 1,59 | 26,85 ± 1,19 | > 0,05 |
| SBP mean 24 hours (mmHg) | 114,69 ± 7,28 | 118,25 ± 6,86 | 0,191 |
| DBP mean 24 hours (mmHg) | 67,38 ± 4,31 | 70,06 ± 4,86 | 0,128 |

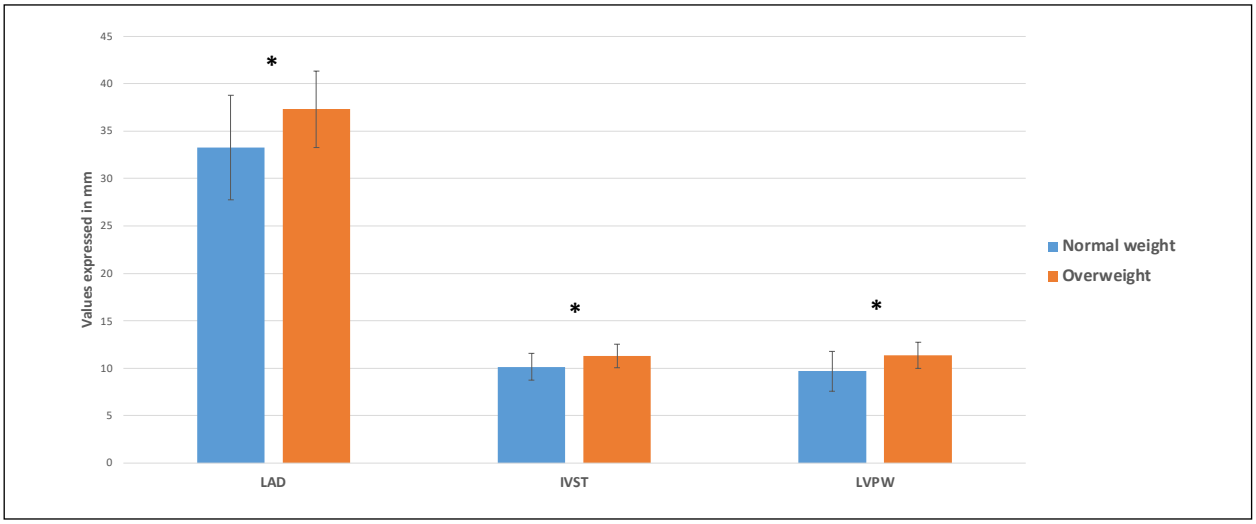


**Figure 1** - *Echocardiographic parameters that have a statistical significance. LAD = left atrial diameter, IVST = interventricular septal thickness, LVPW = left ventricular posterior wall (LVPW).*

## DAPAGLIFLOZIN-INDUCED MYOCARDIAL FLOW RESERVE IMPROVEMENT IS NOT ASSOCIATED WITH HDL ABILITY TO STIMULATE ENDOTHELIAL NITRIC OXIDE PRODUCTION

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**Background.** SGLT2 inhibitors showed controversial results in modulating plasma lipids in clinical trials. HDL-cholesterol slightly increased in most studies but only a few provided evidence on HDL functionality with disappointing results. Otherwise, there is a broad agreement that these drugs provide cardiovascular protection through several mechanisms. Our group demonstrated that dapagliflozin improves myocardial flow reserve (MFR) in type 2 diabetes (T2D) patients with coronary artery disease (CAD). The underlying mechanisms are still unknown, although in vitro studies suggested the involvement of nitric oxide (NO).
**Aim.** To investigate changes in HDL-mediated modulation of NO production with dapagliflozin and whether there is an association with MFR.
**Methods.** 16 CAD-T2D patients were enrolled and randomized 1:1 to dapagliflozin or placebo for 4 weeks. Blood samples were collected before and after treatment for each group. The ability of HDL to stimulate the NO production in endothelial cells was tested in vitro by incubating the human umbilical vein endothelial cells (HUVEC) with apoB-depleted (apoB-D) serum of these patients. The production of NO was assessed by fluorescent assay and results were expressed as fold versus untreated cells.
**Results.** Change in HDL-mediated NO production remains similar in dapagliflozin and placebo group, even after adjustment for confounders. There are no significant correlations between HDL-mediated NO production and MFR both at baseline and after treatment. HDL-cholesterol do not change in both groups, while LDL-cholesterol significantly decreases compared to baseline only in treatment group (p=0.043).
**Conclusions.** In T2D-CAD patients, beneficial effects of dapagliflozin on coronary microcirculation seem to be unrelated to HDL functions. However, HDL capacity to stimulate NO production is not impaired at baseline, thus the effect of drug treatments would be negligible. To conclude, we could assume that HDL-independent molecular pathways are involved in the improvement of MFR in this population.

## LIPOPROTEIN(A) LEVELS AND GENETIC STATUS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Aim.** Lipoprotein(a) – Lp(a) – represents an independent risk factor for cardiovascular diseases that can exacerbate the clinical manifestations in patients with Familial Hypercholesterolemia (FH). FH is a genetic disease caused by an impaired LDL uptake leading to very high LDL-cholesterol levels and premature CVD. We aim to evaluate Lp(a) levels and SNPs in the encoding gene (LPA) in patients with a clinical suspect of FH.
**Patients and Methods.** We analyzed data of 254 patients, including 64 pediatric patients (<16years). Lp(a) levels were measured by an immunonephelometric assay independent from the Kringle IV repeat number; genetic status of patients and LPA SNPs (rs3798220 and rs10455872) were determined by NGS (Devyser FH v2).
**Results.** In the whole population, Lp(a) levels did not differ between patient without (V-) and with pathogenic variants (V+). Among the pediatric group, V- patients showed higher Lp(a) levels [70 (8-100) mg/dL] than the V+ patients [7(13-33) mg/dL, p=0.04). By the analysis of ROC curves, LDL-cholesterol levels and LDL-cholesterol levels corrected for the Lp(a)-cholesterol content, showed a similar capacity to distinguish between V- and V+ patients, both in the total population and in age-based groups. Patients with a Lp(a)-raising allele for one of the two SNPs showed higher Lp(a) levels [89.0(64.5-109.5) mg/dL] than the patients without Lp(a)-raising alleles [13.5(5.3-46.4) mg/dL; p<0.001]. However, 10/141 without Lp(a)-raising alleles showed Lp(a) levels >100 mg/dL, indicating that the presence of SNP is not the only cause of high Lp(a) levels.
**Conclusions.** High Lp(a) levels can contribute to the high LDL-cholesterol levels that led to a clinical suspect of FH, suggesting that Lp(a) levels should be always measured to better define the diagnosis, particularly in pediatric patients, whereas the evaluation of Lp(a)-corrected LDL-cholesterol levels is not essential. The SNP analysis cannot replace the Lp(a) measurement for the identification of patients with very high Lp(a) levels.

## MEAN PLATELET VOLUME (MPV) AS NEW MARKER OF CARDIOVASCULAR RISK IN NEWLY DIAGNOSED HYPERTENSIVE PATIENTS WITH DIFFERENT GLUCOSE HOMEOSTASIS

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The mean platelet volume (MPV) is a measurement of the platelet activity and is considerate a prognostic marker in patients with cardiovascular diseases (CVD). Increased MPV is associated with an increased risk of atherosclerosis and myocardial infarction and can be considered and independent risk factor of death in patients after acute ischemic cardiac incident. It is known that glucose homeostasis alterations are associated with subclinical vascular damage; in particular, the mechanisms at the base of vascular complications of type 2 diabetes (T2DM) already act in the prediabetes phase. Previous studies demonstrated an increase in MPV in patients with T2DM, however it is still unclear whether higher MPV is present in the early phase of diabetes. The aim of the present study was to evaluate MPV values and its possible correlation with arterial stiffness and subclinical myocardial damage, in normal glucose tolerance patients (NGT), in T2DM patients and in those who had prediabetes. We enrolled 400 newly diagnosed hypertensive patients (mean age 60.1 ± 11.9). All patients underwent to Oral Glucose Tolerance test (OGTT). Arterial stiffness (AS) was evaluated with the measurement of carotid-femoral pulse wave velocity (PWV). Echocardiographic recordings were performed using an E-95 Pro ultrasound system. ANOVA test was used to test the differences between groups. A linear correlation analysis was performed to compare values of MPV, PWV and global longitudinal strain (GLS) with different covariates. Variables reaching statistical significance were inserted in a stepwise multivariate linear regression model. We divided the patients into three groups: normoglycemic patients (NGT) (n=179), prediabetic patients (n=137) and newly diagnosed T2DM (n=84). Among three groups, there was an increase in fasting plasma glucose (FPG) ($p<0.0001$), 2h-glucose ($p<0.0001$), fasting plasma insulin (FPI) ($p<0.0001$), 2-h insulin ($p<0.0001$), HbA1c ($p<0.0001$), high sensitivity c reactive protein (hs-CRP) ($p<0.0001$) and a decrease in renal function as demonstrated by e-GFR values ($p<0.0001$). Interestingly, from NGT group to T2DM group there was a raise in MPV value ($p<0.0001$). In particular, higher MPV was found in prediabetic ($p<0.0001$) and T2DM patients ($p<0.0001$) compared to normoglycemic subjects. From linear regression analysis, MPV was significantly and directly correlated with HbA1c ($r=0.251$, $p<0.0001$), hs-CRP ($r=0.120$, $p=0.009$), platelets ($r=0.187$, $p<0.0001$), age ($r=0.107$, $p=0.018$) and inversely correlated with Matsuda/ISI ($r=-0.446$, $p<0.0001$), BMI ($r=-0.165$, $p=0.001$) and e-GFR ($r=-0.397$, $p<0.0001$). Subsequently, stepwise multivariate linear regression model showed that Matsuda/ISI was the major predictor of MPV justifying 19.7% of its variation, e-GFR added another 7.7% and HbA1c and e-GFR added, respectively, 1.7% and 1.1%. In the evaluation of arterial stiffness, PWV resulted significantly and directly correlated with MPV ($r=0.451$, $p<0.0001$), hs-CRP ($r=0.111$, $p=0.015$), LDL cholesterol ($r=0.109$, $p=0.016$) and inversely correlated with e-GFR ($r=-0.152$, $p=0.001$), Matsuda/ISI ($r=-0.382$, $p<0.0001$), HDL cholesterol ($r=-0.140$, $p=0.003$). Stepwise multivariate linear regression model highlighted that MPV was the main predictor of PWV justifying 20.1% ($p<0.0001$) of its variation. Regarding to echocardiographic evaluation, GLS was directly and significant correlated with MPV ($r= 0.383$, $p<0.0001$), E/è ($r=0.409$, $p<0.0001$), left ventricular mass index (LVMI) ($r=0.329$, $p<0.0001$), SBP ($r=0.159$, $p=0.001$), DBP ($r=0.126$, $p=0.006$), hs-CRP ($r=0.100$, $p=0.038$) and inversely correlated with Matsuda/ISI ($r=-0.299$, $p<0.0001$) and e-GFR ($r=-0.112$, $p=0.014$). Stepwise multivariate linear regression model demonstrated that E/è was the main predictor of GLS justifying 16.5% of its variation, MPV added another 8.3%, LVMI added 2.0%. In conclusion, in the present study we highlighted that MPV is significantly increase in newly diagnosed T2DM patients and in early stages of diabetes, indicating that subjects with prediabetes present increased platelets reactivity. Our results suggest that MPV is associated with increased arterial stiffness and subclinical myocardial damage, indicating MPV as a new marker of CV risk.

## REDUCTION IN SERUM LIPOPROTEIN A LEVELS BY APHERESIS AND MEDICAL THERAPY WITH NO REDUCTION IN CARDIOVASCULAR RISK: A CASE REPORT

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**Background.** Lipoprotein A is a cholesterol-rich lipoprotein, similar in structure to LDL, from which it differs in the diversity of its protein component. Circulating levels of Lp(a) are genetically determined. Lp(a) results in pro-osteogenic effects, resulting in endocardial calcium deposition and the subsequent onset of calcific aortic stenosis at an early age; pro-atherogenic effects, and pro-thrombotic effects, related to its direct interaction with proteins in the coagulation cascade.

**Case report.** A 58-year old patient, who underwent PTCA with DES implantation on the right coronary artery in 2004 following an acute myocardial infarction, comes to our observation at the outpatient clinic of rare diseases at Vanvitelli University. At the visit, we pose clinical diangosis of HeHF, later confirmed by genetic analysis. The latter detected the variant c.1646G>A, p.(Gly549Asp), of the LDLR gene (NM_000527.4) in heterozygosity, classified as pathogenic for familial hypercholesterolemia, and the variant rs10455872 of the LPA gene in heterozygosity. We extend the genetic analysis to family members: two out of the three sons have the same mutation as their father. Given the high Lp(a) levels, we decide, in addition to lipid lowering therapy, to undertake apheresis treatment. Despite optimized therapy and control of lipid values, the patient experienced multiple cardiovascular events during our follow-up.

**Conclusions.** It is likely that multiple mechanisms determined by lipoprotein a, probably not directly related to the magnitude of its plasma levels, are involved in the genesis of cardiovascular damage and progression of atherosclerotic disease. Early diagnosis and targeted treatment in these patients are the key to ensure a significant reduction in residual cardiovascular risk. The market entry of two new drugs that act specifically against Lp(a), Olpasiran and Pelacarsen, will likely mark a major breakthrough in the treatment of dyslipidemic patients and provide additional weapons to lower residual cardiovascular risk.

## NEW POTENTIAL BIOMARKERS FOR EARLY CHRONIC KIDNEY DISEASE DIAGNOSIS IN PATIENTS WITH DIFFERENT GLUCOSE TOLERANCE STATUS

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Patients with renal dysfunction present increased risk for cardiovascular (CV) events and dysfunction in hemostatic system, in particular bleeding disorders and thrombosis. In this contest, oxidative stress has a central role in the pathophysiology, development, and complications of Chronic kidney disease (CKD). In addition, recent studies highlighted that endothelial dysfunction plays a significant role in CKD, in particular endocan, a soluble proteoglycan secreted by endothelial cells, and considered a novel biomarker of endothelial dysfunction. Subjects with 1-hour plasma glucose values ≥155 mg/dl (NGT ≥155), detected during oral glucose tolerance test (OGTT), are at increased risk to develop type 2 diabetes mellitus (T2DM) among subjects with normal glucose tolerance test (NGT). Recent observation highlighted that these subjects have a worse cardiometabolic risk profile and an increased risk for CKD. The aim of the current study was to test the role of oxidative stress, platelets activation and endocan levels in renal dysfunction, in NGT <155, NGT ≥155, impaired glucose tolerance (IGT) and T2DM individuals. We enlisted 233 patients (mean age 61.4±10.7). Plasma glucose was measured by the glucose oxidation method and plasma insulin concentration was determined by a chemiluminescence-based assay. Insulin sensitivity was evaluated using the Matsuda index (Matsuda/ISI). Renal function was tested by measurement of estimated glomerular filtration rate (e-GFR) with CKD-Epi formula. The serum levels of platelets activation (Glycoprotein-VI and sP-selectin) oxidative stress biomarkers (8-isoprostane and Nox-2), and endocan were evaluated with ELISA test. ANOVA test was used to test the differences between groups. A linear correlation analysis was performed in the entire study population, to evaluate the possible correlation between e-GFR and different covariates. Variables reaching statistical significance were inserted in a stepwise multivariate linear regression model. According to plasma glucose value during OGTT, subjects were divided into 4 groups: NGT <155, NGT ≥155, IGT and T2DM. Among the study groups, there was a significant increase of SBP (p=0.031), triglyceride (p<0.0001), fasting plasma glucose (FPG) (p<0.0001), 1-h glucose (p<0.0001), 2-h glucose (p<0.0001) during OGTT, as well as fasting insulin (p<0.0001), 1-h insulin (p<0.0001) and 2-h insulin (p<0.0001). As awaited, there was a worsening of insulin sensitivity accounting for the decrease of MATSUDA/ISI (p<0.0001). Moreover, we observed a worsening of the inflammatory profile with the deterioration of glucose tolerance, as attested by hs-CRP values (p<0.0001). In addition, there was a decrease in renal function, as demonstrated by e-GFR values (p<0.0001), in addition there was a statistically significant rise in creatinine (p<0.0001) and azotemia (p=0.002). Of interest, from NGT<155 to T2DM group, there was a statistically significant increase in 8-isoprostane (p<0.0001), Nox-2 (p<0.0001), Glycoprotein-VI (p<0.0001), sP-selectin (p<0.0001) and endocan (p<0.0001) serum levels. Specifically, NGT≥155 patients presented higher serum endocan values compared to NGT<155 (p<0.0001). From the linear correlation analysis, e-GFR resulted significantly and indirectly correlated with 1-h glucose (r=-0.489, p<0.0001), 8-isoprostane (r=-0.473, p<0.0001), Nox-2 (r=-0.479, p<0.0001), endocan (r=-0.476 p<0.0001), Glycoprotein-VI (r=-0.238, p<0.0001), sP-selectin (r=-0.368, p<0.0001), hs-CRP (r=-0.171, p=0.005) and positively correlated with MATSUDA/ISI (r=0.418, p<0.0001). From stepwise multivariate linear regression model 1-h glucose resulted the major predictor of e-GFR justifying 23.6% of its variation, 8-isoprostane and Nox-2 added respectively another 6.0 and 3.2%. In conclusion, our study confirmed the link between 1-hour post-load glucose ≥155 mg/dl during OGTT and the possible increased risk for CKD. In the present study we demonstrated a progressive increase in oxidative stress and platelets activation with the worsening of metabolic profile and significantly early during the progression of CKD. Moreover, our data demonstrated a progressive increase in serum endocan levels, an emerging molecule secreted by endothelial cells, and it seems to be involved in many pathological processes such as CKD.

## MEASURMENT OF TOTAL PLAQUE AREA BY ULTRASOUND: A BETTER TOOL TO ASSESS THE RISK OF CARDIOVASCULAR DISEASE AS COMPARED TO CORONARY CLACIUM

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**Background and Aims.** Carotid plaque burden (CPB) measured as the sum of cross sectional areas of all plaques seen between the clavicle and the angle of the jaw) by ultrasound may be a stronger predictor of cardiovascular than coronary artery calcium score (CAC).

**Methods and Material.** We compared the prediction of cardiovascular events between and coronary artery calcium score performing a systematic review and a meta analysis. We calculated the adjusted hazard ratio of myocardial infarction, stroke and vascular death predicted by CPB and CAC.

**Results.** We selected 7 articles studies assessing the predictive roles of CPB and 17 assessing CAC. In participants without CVD at baseline CPB was a strong predictor of stroke (HR: 1.22, 95%CI: 1.14-1.30), coronary artery disease (1.35, 95%CI: 1.23-1.47), all vascular disease and all-cause mortality (HR: 2.14, 95% CI: 1.50- 3.04). The findings are camparable with CAC (HR: 1.38; 95% CI:1.14-1.66; HR: 1.60; 95%CI: 1.23-2.09; HR: 1.87, 95% CI: 1.16-3. 02, respectively). Likewise, in symptomatic and high-risk patients at baseline, CAC (HR:2.10; 95% CI:1.61-2.73) and CPB (HR: 2.20; 95% CI: 1.57-3.10) were similar predictors of all vascular disease.

**Conclusions.** CPB is predictive of cardiovascular event as CAC, but it has more advantages: it is repeatable because the absence of exposure radiation, less expensive and better predictive of cardiovascular risk in women and young. It is, furthermore, more powerful in monitoring atherosclerosis progression and its medical therapy.

## ASSOCIATION BETWEEN SEVERITY OF LIVER AND CARDIOVASCULAR DAMAGE IN PATIENTS WITH METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD) ACCORDING TO CARDIOVASCULAR RISK CATEGORIES

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**Background.** Non-alcoholic fatty liver disease (NAFLD) has been associated with several cardiovascular (CV) risk factors including obesity, dyslipidemia, hyperglycemia, hypertension, and an increased risk for liver and CV disease (CVD) morbidity and mortality has been described. Recently, to better define NAFLD a new nomenclature metabolic dysfunction associated steatotic liver disease (MASLD) has been proposed, combining liver steatosis with at least one cardiometabolic factor. In 2021, the European Society of Cardiology (ESC) redefined the CV risk and identified three categories of risk (low, high, and very high).
**Aim.** To evaluate in MASLD patients the severity of metabolic comorbidities and liver damage according to the new definition of CV risk.
**Methods.** 287 MASLD patients, fully characterized for metabolic, liver, and CV features, were divided into 3 groups according to the degree of CV risk. Liver fibrosis was assessed through liver stiffness measurement (LSM) and confirmed by histology in 29. CV damage was defined by carotid plaques or carotid intima-media thickness (cIMT) ≥0.9mm by ultrasound. Epicardial fat thickness (EFT) was evaluated by echocardiography.
**Results.** With rising CV risk a significant increase in BMI (29±4.8 vs 31±4.4 kg/m$^2$, p<0.001), obesity (33% vs 58%, p<0.001), liver fibrosis (4.7±2.0 vs 5.2±2.2 kPa, p=0.02), advanced histological liver fibrosis (F3-4) (0% vs 17%, p=0.04), EFT (7.3±2.8 vs 8.3±3.0 mm, p=0.04), diabetes (7 vs 23%, p=0.004) and hypertension (39 vs 63%, p=0.003) was observed. As expected, carotid plaques and cIMT progressively increased from low to high-very high CV risk (p<0.001, and p=0.0003, respectively). At multivariate analysis, adjusted for age, sex, and all metabolic parameters, LSM was independently associated with the highest CV risk (OR 1.2, 95% C.I. 1.01-1.31).
**Conclusion.** The association between cardiometabolic factors and severity of liver fibrosis exposes patients with MASLD to an increased CV risk and should be well evaluated in all patients.

## ANKLE-BRACHIAL INDEX IS REDUCED IN NON-ASTHMA EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

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**Introduction.** Eosinophilic Granulomatosis with polyangiitis (EGPA) is a vasculitis of small vessels characterized by eosinophil infiltration and consequent wall damage. The disease is characterized by asthma, pulmonary infiltration, skin purpura and neurological manifestations. Heart failure is one of the main comorbidity inducing a fatal outcome. Asthma is known relating to an increase in vascular stiffness. Despite it is a vessel wall disease, no data are available on vascular stiffness in EGPA.
**Aim.** With this study we would to evaluate if EGPA may induce a change in vascular stiffness and if it is related to a cardiovascular risk.
**Methods.** We include 19 caucasian EPGA (8 males/11 females, aged 55.26±10.97 y). Patients are underwent by physical examination and routine lab tests. None report an history of cardiovascular disease or lipid lowering treatment. All patients perform Ankle Brachial Index (ABI), complete heart ultrasound, and Doppler ultrasound to evaluate the presence of carotid atherosclerosis and intima-media thickness (IMT). ACC ASCVD Risk Score and ESC SCORE2/2-OP estimate the CV risk. Patients are divided into those who presented asthma (Group 1, 1 male / 7 females, aged 53.12±13.92 y) and those who not (Group 2, 7 males / 4 females, aged 56.82±7.87 y).
**Results.** No difference there are in BMI, weight and height of the patients. Group 1 presents a decreased HDL (p=0.040). No difference there is in blood glucose, triglycerides, total and LDL cholesterol. DBP (p=0.021) but not SBP decrease in Group 1. No difference there is in ASCVD or SCORE risk evaluation. ABI increase in group 1 (p=0.004) despite no difference there is in IMT. All patients present a Left ventricle mass above the normal values but no difference there is between the groups. In group 1 trans-mitral velocity A increases (p=0.041), while velocity E (p=0.049) and velocity e' (p=0.025) decrease, resulting increase in diastolic dysfunction (p=0.048). Finally, evaluating all population, HDL directly relates to E/A ratio (r=0.73, p=0.016) and to eosinophils absolute count (r=0.76; p=0.016), while ABI inversely relates to E/A ratio (r= -0.55; p=0.001) and to relative wall thickness (RWT, r=-0.33, p=0.049).
**Conclusion.** Differently to data described in patients with asthma alone, EGPA patients with asthma appear to have an enhanced vascular compliance. However, these patients present a worsen heart damage. HDL relates to eosinophils and may play a role in these complex phenomena.

## RESPONSE TO PHARMACOLOGICAL THERAPY IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA CLINICALLY SUSPECTED AND/OR GENETICALLY CONFIRMED

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**Background.** Familial hypercholesterolemia (FH) is a frequent genetic pathology with autosomal dominant or recessive Mendelian transmission, characterized by the plasma accumulation of LDL, which is responsible for premature atherosclerosis. In recent decades, the classification of suspected cases through careful anamnesis and possible confirmation with genetic tests has made it possible to intercept numerous cases of FH early, allowing for valid management of the pathology. At the same time, the advent of new bio-technological lipid-lowering drugs has made new increasingly effective therapeutic strategies available.
**Aim.** To evaluate the efficacy to lipid-lowering therapy (LLT) (monotherapy or Fixed Dose Combination therapy) and the prevalence of early cardiovascular events based on the patient's genotypic characteristics, to converge the choices of clinical practice towards more targeted and personalized interventions.
**Methods.** Out of 2437 patients belonging to the Regional Referral Outpatient Lipid Clinic of the SS. Annunziata Hospital in Chieti between 2013 and 2021, 145 patients with a phenotype suggestive of FH were selected and called to perform genetic examination looking for mutations in the main genes associated with FH (LIPIGEN Project, SISA Foundation).
**Results.** 50 patients tested negative (FH-) and 95 positive (FH+); of the latter, 71 were found to be carriers of a single mutation and 24 were compound heterozygotes. Three of them were carriers of two different mutations, both of certain pathogenicity as described in the genetic (Lipid InCode or previous Labs) report. 70% of the mutations detected concern the LDLR gene, 13% APOB, 8% PCSK9 and the remaining 9% concerned other genes (LDLRAP1, LIPA, APOE) globally. From our data, it seems that FH+ patients have higher pre-therapy LDL values and lower achievement of their LDL target compared to FH- patients. The response to LLT varies considerably depending on the specific mutation present: in particular - as expected and simply explicable - a worse response to therapy has been found for compound heterozygotes compared to single mutation carriers, while in patients carrying a PCSK9 mutation (evidently Gain of Function) there is an excellent response to therapy with PCSK9i. As regards the prevalence of early cardiovascular events, it is higher in the group of double heterozygotes for LDLR mutations, as well as in the case of the two patients carrying of a APOE mutation. Finally, regarding a gender comparison, our data have shown that female, both in FH+ and FH- groups, seems to have pre-treatment LDL values that tend to be higher than males and have more difficult to achieve their target than male.
**Conclusions.** On the basis of these results, and because we are going towards an increasingly individualized therapeutic approach, larger studies are already ongoing (see LIPIGEN Study, of which these results represent only a monocentric sample) and further studies will be necessary for the identification of new pathogenic mutations for FH and which possibly influencing the response to LLT.

## LYSOSOMAL ACID LIPASE ACTIVITY PREDICTS LIVER FIBROSIS PROGRESSION IN PATIENTS WITH METABOLIC ASSOCIATED STEATOTIC LIVER DISEASE

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**Background and Aim.** The definition of Metabolic Associated Steatotic Liver Disease (MASLD) was proposed by an international expert panel to identify fatty liver conditions associated with metabolic disorders and replace the definition of Non-Alcoholic Fatty Liver Disease (NAFLD). MASLD is characterized by liver steatosis, defined by imaging or liver biopsy, and is associated with at least one metabolic disorder. The presence of metabolic disorders is established in the presence of at least one of the metabolic syndrome criteria (BMI ≥25 kg/m$^2$ can also be used instead of waist circumference). Lysosomal Acid Lipase (LAL) is involved in lipid metabolism and is responsible for hydrolyzing cholesteryl esters and triglycerides. Genetically reduced LAL activity induces cholesterol ester accumulation in many organs, as observed in Wolman and cholesterol ester storage diseases. Previous study demonstrated a non-genetic reduction of LAL activity in NAFLD and in NAFLD-related cirrhosis. Currently, there are no prospective data on the association between LAL activity and MASLD progression. For these reasons, this study aimed to evaluate the association between LAL activity and liver fibrosis progression in MASLD.
**Methods and Results.** MASLD criteria were applied to a prospective ongoing cohort (The Plinio Study) of dysmetabolic non-cirrhotic patients investigated for the presence of liver steatosis. All patients were negative for alcohol abuse and the presence of viral, autoimmune, or iatrogenic hepatitis. In the analysis were included only patients with basal LAL activity measurement and with at least two FIB4 evaluations during the follow-up (n=272). LAL activity was measured with dried blood spot technique. Fib4 was calculated according to guidelines. MASLD fibrosis progression was defined as changes in Fib4 values from negative (<1.3 if patient was <65years or <2.0 if patient was ≥65 years) to indeterminate or positive. New cirrhosis incidence was defined as changes in Fib4 from ≤2.67 to >2.67. A total of 272 MASLD patients were recruited (mean age 55.6±10.4 years; 36.4% women). At baseline LAL and FIB-4 were inversely associated (rS=-0.126, p=0.038). Median follow-up time was 70.5 months (1720 patient-years). During the follow-up 49 patients showed fibrosis progression, and 10 patients passed from Fib4 ≤2.67 to >2.67. Patients with liver fibrosis worsening had lower basal LAL activity (0.90 [0.26-2.02] vs 0.72 [0.24-2.01]; p=0.024); similar results were found in patients who developed new cirrhosis (0.90 [0.26-2.02] vs 0,66 [0.32-1.57]; p=0.023) at follow-up. Patients were distributed according to LAL activity tertiles. Subjects with lower enzymatic activity had higher risk to develop liver disease progression (I vs III tertile log-rank test p=0.025; I vs II+III tertile log-rank test p=0.008) and hepatic cirrhosis (I vs II+III tertile log-rank test p=0.028).At the multivariable Cox regression analyses, the lower LAL activity tertile was associated with liver fibrosis worsening (HR [hazard ratio] 2.11; 95% CI, 1.19-3.73; p=0.011) and with cirrhosis incidence (HR, 5.39; 95% CI, 1.22-23.90; p=0.027), independently from age, female sex, BMI, diabetes, and basal Fib4 class.
**Conclusions.** LAL activity was associated with liver fibrosis worsening and cirrhosis onset in MASLD patients. Our results suggest that LAL activity may be an easy and useful marker to identify a subgroup of patients at higher risk of liver fibrosis progression. Further studies need to be conducted on a larger population to confirm the presented data and to establish if LAL activity could represent a possible therapeutic target in MASLD.

## IMPACT OF DIETARY CHOLINE ON HOMOCYSTEINE METABOLISM IN ATHEROSCLEROSIS PRONE MICE

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**Aim.** Scientific evidence revealed that a positive correlation exists between cardiovascular risk and plasma levels of TMAO, a product of dietary choline metabolism. This study was aimed at investigating whether dietary choline affects additional metabolic pathways besides that leading to TMAO production.
**Methods.** Ten-week-old EKO female mice were fed for 16 weeks two standard rodent diets differing for a low (0.09%) or high (1.2%) choline content. Atherosclerosis development was quantified at the aortic sinus, targeted plasma metabolomic and hepatic gene expression were performed. Additionally, in vitro experiments on HepG2 cells were set up to elucidate the mechanism by which choline alters plasma metabolome.
**Results.** High choline intake was associated with greater atherosclerosis development and increased plasma levels of TMAO. Interestingly, high choline feeding was associated with lower plasma levels of homocysteine and a concomitant increase of its related metabolites, methionine, sarcosine and glycine. Hepatic gene expression of Aldh7a1, Slc44a1, Sardh and Gnmt was increased in EKO mice fed high-choline diet, supporting the metabolic findings. In vitro experiments showed that several pathways are devoted to homocysteine metabolism and can be mutually regulated by acting on enzymes belonging to different synthetic routes.
**Conclusions.** Our data confirm that an increased dietary intake of choline worsens atherosclerosis burden and leads to increased plasma TMAO levels. Interestingly, choline intake also modulates metabolic processes affecting plasma concentrations of homocysteine as well as methionine, sarcosine, and glycine. These observations offer new insights into the understanding of how choline might influence atherosclerosis development and modify cardiovascular risk.

## TYPE 2 DIABETES MELLITUS IS ASSOCIATED WITH INCREASED RISKS OF LOW HANDGRIP STRENGTH AND SARCOPENIA IN ADULTS

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**Background.** Type 2 diabetes mellitus (T2DM) is steadily increasing worldwide. T2DM has beenidentified as a contributing factor to sarcopenia. Recent evidence, on Asian populations, suggeststhat individuals with diabetes have a higher risk of developing sarcopenia than those withoutdiabetes. However, there has been limited research conducted in European populations. Theprimary objective was to determine the prevalence of sarcopenia and investigate the associationsbetween T2DM and sarcopenia in European adults.
**Methods.** This retrospective cross-sectional study included 356 individuals aged ≥50 years. Bioelectrical impedance analysis andhandgrip dynamometry were employed to measure appendicular skeletalmuscle mass and handgrip strength (HGS), respectively. Sarcopenia wasdefined according to the criteria outlined in the EWGSOP2 guidelines,which involve the presence of low HGS (< 16 kg for women and < 27 kg formen) combined with low appendicular skeletal muscle mass (< 15 kg inwomen and < 20 kg in men). T2DM was diagnosed based on a fasting bloodglucose concentration of ≥126 mg/dL or the administration ofantidiabetic treatment.
**Results.** The mean age of the participants was 69±7 years, with 39% being male. The prevalenceof T2DM was 22%, and 12% were treated with hypoglycemic drugs. The overall prevalence ofsarcopenia among European participants was 9%. Participants with T2DM exhibited a significantlyhigher prevalence of low HGS (32% vs. 18%, p-adjusted=0.008), as well as sarcopenia (15% vs. 7%, p-adjusted=0.02) compared to those without T2DM. Multinomial logistic regression analysisrevealed that T2DM was associated with increased odds of having low HGS (OR=2.60; 95% CI=0.99-6.87) and sarcopenia (OR=6.38, 95% CI=1.63-24.9).
**Conclusions.** Older European adults with diabetes face a significantly higher risk of developinglow HGS and sarcopenia compared to their non-diabetic counterparts. This study confirms thatT2DM is an important influencing factor in the development of sarcopenia.

## ELEVATED PULSE-WAVE VELOCITY IS AN ADDITIONAL RISK IN PATIENTS WITHOUT CARDIAC ORGAN DAMAGE

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**Background.** Vascular stiffness is a well-known parameter to evaluate organ damage (OD) in high and very high cardiovascular risk patients, although very few doctors are currently using in their practice. Aim of this study was to evaluate how vascular stiffness by itself may change the evaluation in OD.
**Methods.** We evaluated 324 hypertensives (147 males, 62.80±11.56 years) under treatment since 1 year. All patients, free by kidney disease, underwent 2-D echocardiography to calculate left ventricle mass indexed (per heigh t2.7). Patients were divided in those with who presented heart hypertrophy (Group 1, 172 patients 91 males, 66.38±9.80 years) and those who not (Group 2, 152 patients 56 males, 58.75±12.09 years). Than we split Group 2 in those with pulse wave velocity (PWV) ≥10 m/s (Group 2A, 46 patients 14 males, 63.41±9.52 years) and those <10 m/s (Group 2B, 106 patients 37 males, 55.79±12.44 years). Cardiovascular risk score (CVRS) was evaluated using the ESC chart SCORE2/SCORE2-OP.
**Results.** Group 1 presented an increase in SBP, weight, BMI and waist circumference compared to Group 2 and Group 2B but not to Group 2A ($p<0.05$ each) while no difference was found in DBP and HR. No difference was found also in total Cholesterol, HDL and LDL-C. On the contrary, Triglycerides and blood glucose ($p<0.05$, both) were higher in Group 1 despite risk of NAFLD, evaluated as TyG score, was similar between the groups. Moreover, only blood glucose was increased in Group 2A compared to 2B ($p<0.05$). CVRS was higher in group 1 and group 2A compared to Group 2 and 2B ($p<0.05$).
**Conclusion.** In conclusion, PWV allowed to identify 15% more patients who present an OD, and consequently, with a cardiovascular risk and metabolic profile comparable to patients with patients with heart hypertrophy. PWV should be routinely employed to evaluate hypertensives since the early stages of the disease.

## FUNCTIONAL CHARACTERIZATION OF UNCERTAIN SIGNIFICANCE VARIANTS (VUS) IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA (FH) THROUGH THE APPLICATION OF FLOW CYTOMETRY

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**Background.** Familial hypercholesterolemia (FH) is an autosomal disorder of lipid metabolism presenting with increased cardiovascular risk. LDLR mutations are the cause of disease in 90% of the cases but the majority of variants lacks functional evidence to evaluate their pathogenicity and are classified as variant of uncertain significance (VUS). This generates uncertain results making the definitive diagnosis of FH difficult.
**Aim.** We aim to assess the functionality of VUS LDLR variants identified in FH patients by using flow-cytometry technique.
**Methods.** Peripheral blood mononuclear cells (PBMCs) from patients carrying LDLR VUS variants were isolated from blood samples using a density gradient method and stimulated with CD3/CD28 beads in a medium containing a lipoprotein deficient serum. The LDLR activity (expression on cell surface, LDL binding and uptake) was measured through flow cytometry. PBMCs from normocholesterolemic subjects (HD) and FH carriers positive to one known pathogenic variant were used as negative and positive controls. Results were expressed, in percentage, as ratio between mean fluorescence of patients and negative controls cells'.
**Results.** Of the 6 VUS variants tested (n=6 FH carriers), the c.1860G>T showed a reduced binding (HD: 100%, c.1860G>T: 38%), uptake (HD: 100%, c.1860G>T: 13%) and expression of LDL receptor (HD: 100%, c.1860G>T: 26%). Conversely, the c.(-97)G>A presented reduced binding (HD: 100%, c.(-97)G>A: 65%) and uptake (HD: 100%, c.(-97)G>A: 76%) but almost similar LDLR expression compared to HD cells. The c.1530_1532del had normal binding (HD:100%, c.1530_1532 del: 111%) and uptake (HD:100%, c.1530_1532del: 102%) but reduced LDLR expression (HD:100%, c.1530_1532del: 68%). Finally, for 3 of the 6 VUS variants tested (c.2282C>T, c.929T>A and c.941-20C>T) expression, binding and uptake values were equal to those observed in HD cells.
**Conclusions.** We have functionally profile six VUS variants in the LDLR gene. These results suggest the importance to conduct functional tests to improve the molecular diagnosis of FH.

## HEPATOCYTE MITOCHONDRIAL FUSION IMPAIRMENT AFFECTS BILE ACIDS CONJUGATION AND NAFL DEVELOPMENT

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**Aims.** Mitochondria are plastic organelles that continuously undergo biogenesis, fusion, fission, and mitophagy to control cellular energy metabolism, calcium homeostasis, hormones, sterols, and bile acids (BAs) synthesis. On these premises, we test the impact of OPA1 deficiency, a key protein of the inner mitochondria membrane fusion, on bile acids production and the following effects on lipid absorption and the development of metabolic dysfunction.
**Methods and Results.** Male mice selectively lacking the critical protein involved in inner mitochondrial fusion, OPA1, (OPA1D-Hep) on a high-fat diet (HFD) for 20 weeks. OPA1DHep mice were protected from the development of hepatic steatosis and obesity because of reduced lipid absorption; a profile which was accompanied by an increased respiratory exchange ratio in vivo, suggesting a preference for carbohydrates in OPA1DHep in agreement with the defect in mitochondrial fusion. At the molecular level, this phenotype emerged as a consequence of poor mitochondrial-peroxisome-ER tethering in OPA1 deficient hepatocytes thus impairing bile acid conjugation and its release in the bile, thus impacting lipid absorption from the diet.
**Conclusion.** Hepatic Opa1 deficiency protects mice from HFD-induced metabolic dysfunction resulting in a reduction of lipid metabolism as a consequence of an alteration in bile acids production.

## STATIN THERAPY IN CRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION: A POST-HOC ANALYSIS OF T.O.S.C.A. REGISTRY

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Statin Treatment (ST) reduces the incidence of Miocardial Infarction (MI), cardiovascular (CV) mortality and first occurrence of Heart Failure (HF) in patients with coronary artery disease (CAD). However, the effect of ST on MI and CV mortality in individuals with known HF is not clear. While observational studies reported clinical benefit of ST, 2 large randomized controlled trials showed no effect on CV mortality and incidence of MI in patients with HF and Reduced Ejection of Fraction (HFrEF), with only a small reduction of MI only in those with an Ischemic Etiology (IE). The aim of our study was to evaluate if ST was associated with all-cause mortality (ACM) or hospitalization for CV causes (HCV) in HFrEF. We conducted a post-hoc analysis of T.O.S.C.A. Registry, an observational multicentric prospective study enrolling patients with HF with FE ≤45% on stable medical therapy and without history of recent decompensation or acute coronary syndrome. Information on ST, clinical, instrumental and laboratory data were used for the analysis. Primary outcome was a composite of ACM and HCV. Among the 377 subjects analyzed, 66% were on ST. Mean age was 63 years and 81% were males. The IE was reported in 52% of subjects and 60,5% were in ST. During 48-month follow-up, 328 subjects were hospitalized for CV reasons or died, of those 221 in patients in ST. ST did not influence primary outcome [HR 0.91 (0.68-1.21); p=0.51]. At the univariate analysis, only age, NYHA class, EF and renal function were significantly associated with the primary outcome, while IE and LDL-Cholesterol levels were not. In conclusion, ST was not associated with a reduction of ACM or HCV in subjects with HFrEF. Further well-powered studies are needed to evaluate whether a clinical net benefit of ST exists in HF.

## ROLE OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND CORRELATION WITH VASCULAR STIFFNESS AND ORGAN DAMAGE

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**Background.** Atherosclerosis is a chronic cardiovascular disease. High plasma concentration of LDL is the main risk factor but the role of inflammation in all the stages become recently evident. Atherosclerosis and lipid metabolism change according to gender differences. Several possible markers of endothelial dysfunction and vascular stiffness have been identified, including clinical-instrumental and biochemical markers of inflammation and/or fibrosis (i.e., TNF-alpha, prostaglandin, L-Arginine/Asymmetric dimethylarginine - ADMA). A common mechanism identified in the pathogenesis of numerous CVD is endothelial dysfunction that correlates with circulating endothelial progenitor cells (EPCs). These cells are recruited from the bone marrow in response to vascular damage or tissue ischemia. In the peripheral blood, EPCs contribute to reendothelialization and neovascularization, acting as positive regulators of hemostasis and vascular integrity. A reduction in their number or function has been correlated to progression of CVD and MACE. Our project aims to assess the role of EPCs and L-ARG/ADMA in arterial stiffness and atherosclerosis.
**Materials and Methods.** Our study is a prospective experimental investigation, enrolling all patients with cardiovascular disease. Patients will undergo routine hematological and biochemical tests as well as instrumental examinations in accordance with good clinical practice. The enrolled patients will undergo a series of clinical and instrumental examinations (ABI,PWV). In in vitro experiments, EPCs will be isolated from PBMCs and functional assays will be performed. Also, Proteome Profiler Target Analytes study and ELISA assay (L-Arg/ADMA) will be performed.
**Results.** There was a direct correlation between SCORE2/2-OP and PWV (r=0.70; p<0.0001). Preliminary data indicated a correlation between L-Arg/ADMA to SCORE2/2OP (r=0.36, p=0.04), Total Cholesterol (r=0.75, p=0.033) and LDL (r=0.75, p=0.031), as well as EPCs and their migration correlates to a change in folate cycle (P<0.007).
**Conclusions.** Preliminary data suggests the importance of EPCs as specific read-out of cell activation and tissue damage in response to cardiovascular risk factors.

## CORRELATION BETWEEN EPICARDIAL FAT AND HEPATIC DAMAGE IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

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**Background and Study Objective.** Human Immunodeficiency Virus (HIV) type 1 infection is associated with hepatic and cardiovascular comorbidities. Growing data show that epicardial fat may be used as a marker of cardiovascular risk, but data on people with HIV are scarce. We evaluated the relationship of epicardial fat with metabolic alterations and liver damage in this high-risk population.
**Methods.** 88 patients on effective antiretroviral treatment (undetectable viral load) were enrolled. Rest echocardiography with epicardial fat thickness measurement was performed. Liver steatosis degree by Hamaguchi criteria and liver stiffness grade by elastosonography (Acoustic Radiation Force Impulse -ARFI- Tecnique) were quantified. Patients were divided into 2 groups according to the median of epicardial fat.
**Results.** Mean age was 53 years and 29% were women. Patients with epicardial fat above the median had a worse metabolic profile (higher Body Mass Index and waist circumference, higher insulin resistance), larger antero-posterior left atrial diameter and worse diastolic function than those below the median. Increased epicardial fat directly correlated with liver damage, such as Hamaguchi score (rS=0.283 p=0.008), and ARFI (rS=0.344 p=0.001). Higher liver stiffness values were associated with indicators of disease history (lower CD4+ T lymphocyte nadir, longer duration of infection) and the inflammatory state (higher Reactive C Protein levels). Increased fibrotic burden was associated with increased atrial chamber size and a worse diastolic function profile.
**Conclusions.** Our data show a correlation between increased epicardial fat, liver damage and metabolic profile in people living with HIV. Measurement of epicardial fat may identify patients at higher risk of metabolic dysfunction and liver damage who may benefit from early and intensive monitoring and cardiovascular risk factors management.

## EVALUATION OF HDL-BOUND LONG NON-CODING RNAS IN SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Aims.** Long non-coding RNAs (lncRNAs) could be attractive circulating biomarkers for cardiovascular risk stratification in subjects at high ASCVD risk such as familial hypercholesterolemia (FH). Our aim was to investigate the presence of lncRNAs carried by HDL in FH subjects and to evaluate the associations of HDL-lncRNAs with lipoproteins and mechanical vascular impairment assessed by pulse wave velocity (PWV).
**Methods.** This was a retrospective observational study involving 94 FH subjects on statin treatment. Biochemical assays, HDL purification, lncRNA and PWV analyses were performed in all subiects.
**Results.** LncRNA HIF1A-AS2, LASER and LEXIS were expressed in HDL; moreover, HDL- lncRNA LEXIS was associated with Lp(a) plasma levels ($p < 0.01$). In a secondary analysis, the study population was stratified into two groups based on the Lp(a) median value. The High-Lp(a) group exhibited a significant increase of PWV compared to the Low-Lp(a) group ($9.23 \pm 0.61$ vs $7.67 \pm 0.56$, $p < 0.01$). While similar expressions of HDL-lncRNA HIFIA-AS2 and LASER were found in the two groups, the High-Lp(a) group exhibited a significant downregulation of HDL-lncRNA LEXIS compared to the Low-Lp(a) group (fold change -4.4, $p<0.0001$). Finally, Lp(a) and HDL-lncRNA LEXIS were associated with PWV (for Lp(a) $p<0.01$; for HDL-lncRNA LEXIS $p < 0.05$).
**Conclusions.** LncRNA HIFIA-AS2, LASER and LEXIS were expressed in HDL; moreover, significant relationships of HDL-lncRNA LEXIS with Lp(a) levels and PWV were found. Our study suggests that HDL-lncRNA LEXIS may be useful to better identify FH subjects with more pronounced vascular damage.

## TREATMENT WITH BEMPEDOIC ACID IN PATIENTS WITH STATIN INTOLERANCE: PRELIMINARY REPORT OF A SINGLE CENTRE REAL WORLD STUDY

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**Background.** Statins are a milestone in treatment of hypercholesterolemia. Introduction of Ezetimibe and PCSK-9 inhibitors dramatically changed therapeutic scenario. Nevertheless, LDL-C target is not always achieved particularly in patients with familial hypercholesterolemia, very high cardiovascular risk or with statin intolerance. For these patients is now available the therapy with Bempedoic acid. By inhibiting ATP–citrate lyase, Bempedoic acid triggers the upregulation of low-density lipoprotein receptor expression in the liver, resulting in increased clearance of low-density lipoprotein particles and LDL-C reduction.
**Methods.** We conducted a real-world study to assess efficacy and safety of Bempedoic acid in patients with statin intolerance. We enrolled patients with hypercholesterolemia and statin intolerance for muscle-related symptoms eligible to start therapy with Bempedoic acid. We evaluated lipid profile, liver and muscles enzyme at baseline and after 4 weeks of bempedoic acid treatment.
**Results.** We preliminary enrolled 12 patients. The mean age of these patients was 57 years; no patient reported cardiovascular event but 66% were hypertensive, 42% had carotid atherosclerosis, and 16% were smokers. After a 4week treatment with Bempedoic acid, we observed a significant reduction in LDL-C levels (from 133.0 $8 \pm 26.74$ mg/dL to $79.16 \pm 22.64$ mg/dL, $p<0.001$). The LDL-C reduction over 25% was achieved in 92% of patients with a mean reduction of 39%. None reported any adverse event such as elevation of liver and muscular enzymes.
**Conclusions.** In this preliminary report we showed that Bempedoic acid could be a valuable treatment option for patients with statin intolerance not reaching LDL-C target despite a maximally tolerated lipid-lowering treatment. Although, patients'enrollment in our center is at an early stage, we have achieved excellent results at the first follow up in the absence of adverse events.

## ROLE OF OPA1-MEDIATED MITOCHONDRIAL DYNAMIC IN KUPFFER CELLS ON SYSTEMIC METABOLISM

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**Background and Aim.** Kupffer cells are hepatic resident macrophages that are essential for liver physiology and contribute to the development of Non-Alcoholic Fatty Liver Disease (NAFLD). OPA1 is a mitochondrial-shaping protein whose activity promotes mitochondrial fusion and positive modulation of oxidative phosphorylation. Since mitochondria are critical for the energy requirements of Kupffer Cells, this project aims to study how OPA1-driven alteration of mitochondrial dynamism might impact systemic lipid metabolism and immune-metabolic response.
**Methods.** Mice with selective OPA1 deficiency in Kupffer cells were fed either a low-fat diet (chow diet) or a high-fat diet for 20 weeks. The metabolic phenotype was assessed by in vivo indirect calorimetry, measurement of plasma and tissue lipid profile, glucose and insulin sensitivity assays, and immunophenotype characterization. Single cell RNA sequencing was also performed to profile with higher resolution the impact of OPA1 deficiency on Kupffer Cell function and cross talk with other liver cells.
**Results.** Under chow diet conditions, mice selectively lacking OPA1 in Kupffer cells showed lower energy expenditure, lower O2 consumption and lower CO2 production, despite a comparable respiratory exchange ratio. The systemic immune profile was comparable between the two groups, with expected differences between the low- and high-fat diet conditions. During the high-fat diet, no significant differences were observed between the two groups in plasma cholesterol and triglyceride levels and in glucose and insulin sensitivity tests, while a significant reduction in liver fibrosis was observed through liver histology.
**Conclusions.** These preliminary data suggest that OPA1-mediated mitochondrial function in Kupffer Cells affects the systemic metabolic response differently under low- or high-fat diet conditions, where systemic metabolism is comparable, but the loss of OPA1 appears to influence the progression of NAFLD through a reduction in liver fibrosis. Ongoing studies aim to understand the molecular mechanisms underlying these different immune-metabolic responses.

## IDENTIFICATION OF CREB3L3 MUTATIONS IN PATIENTS WITH HYPERTRIGLICERIDEMIA

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**Introduction.** Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. According to recent guidelines fasting TG plasma levels <1,7 mmol/l (150 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. Very severe HTG, defined at TG >20 mmol/L (>1770 mg/dL) is much more rare (prevalence 0.014%). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations in canonical genes involved in triglyceride metabolism: LPL, APOC2, APOA5, GPIHBP1, and LMF1. Mutations in CRE-binding protein 3-like 3 (CREB3L3) and glucokinase regulator (GCKR) have been associated to dominant forms of familial hypertriglyceridemia.
**Materials and Methods.** We performed targeted Next Generation Sequencing (NGS) analysis to study the coding regions and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism in 19 patients with moderate, severe and very severe hypertriglyceridemia.
**Results and Conclusion.** A total of five patients were found to be carriers of variants in CREB3L3 gene in heterozygosity. Three patients with heterogenous phenotype (moderate, severe and very severe HTG) were found to be carriers of a nonsense variant (c.610C>T - p.Arg204Ter). A patient with severe HTG was carrier of a previously reported pathogenic mutation (c.718G>A p. Glu240Lys). Another one with moderate HTG was found to be carrier of a loss of function mutation (c.732_733insG - p.Lys-245GlufsTer130). Our results indicate that CREB3L3 gene does not appear to be associated only with moderate HTG phenotype typically seen in autosomal dominant hypertriglyceridemia. Further studies are needed for elucidate the genotype-phenotype correlation in these patients.

## IMPACT OF THE POLYGENIC SCORES ON THE PREDISPOSITION TO HIGH LDL-C LEVELS IN PATIENTS WITH FAMILIAL HYPERCOLESTEROLEMIA

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**Aim.** Familial Hypercholesterolemia (FH) is a frequent genetic disease characterized by high levels of LDL-cholesterol (LDL-c) and increased cardiovascular risk; it is mainly caused by pathogenic variants in three genes (LDLR, APOB and PCSK9). In hypercholesterolemic patients without FH-causative variants, the increased LDL-c levels could have a polygenic cause. We aim to evaluate the role of two polygenic scores proposed by Talmud et al. 2013 (12-SNPs score) and Futema et al. 2014 (6-SNPs score) in FH-suspected patients.

**Methods.** We performed genetic analysis of 544 patients with a clinical suspect of FH by Next Generation Sequencing (NGS) that allows both to sequence the main causative genes of FH and to characterize the SNPs to calculate the genetic scores associated with high LDL-c levels.

**Results.** Considering the proposed cut-off for both scores, no differences of score positivity were observed between patients with (FH/V+) or without pathogenic variants (FH/V-). On the other hand, considering the score values, higher values of the 12-SNPs score were observed in FH/V- patients (1.002 (0.905-1.098)) than in FH/V+ patients (0.989 (0.847-1.080); p=0.028). Stratifying patients according to LDL-c levels, this difference remain significant in the range of LDL-c 150-250 mg/dL. Values of the 12-SNPs score are directly correlated with LDL-c levels in FH/V+ patients (p=0.032). This association remain significant at multivariate linear regression independently from other factor usually impacting LDL-c levels, i.e., sex, age and presence of pathogenic variants (beta-coefficient= 0.167 p=0.033).

**Conclusions.** Despite FH/V- patients have higher 12-SNPs score values than FH/V+ patients, the proposed cut-offs are not useful to distinguish the two groups, suggesting that they should be improved. The 12-SNPs score can be considered to evaluate the predisposition to high LDL-c levels in hypercholesterolemic patients with a genetic diagnosis of FH.

## EFFECT OF COENZYME Q10 ON PHYSICAL PERFORMANCE IN ELDERLY PATIENTS WITH STATIN-ASSOCIATED ASTHENIA: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIAL

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This double-blind, randomized, placebo-controlled clinical study aimed to investigate the effect of chronic dietary supplementation with coenzyme Q10 (CoQ10) phytosome on physical performance in older adults with statin-associated asthenia. Participants were randomized to receive daily either 2 indistinguishable pills of placebo or 150 mg CoQ10 phytosome (i.e. 300 mg CoQ10 phytosome per day, equivalent to 60 mg CoQ10; Ubiqsome®). Asthenia and handgrip strength (HGs), 1-minute sit-to-stand (STS) repetitions and 2-minute steps (2MST) were performed at baseline and at 8-week follow-up. After the first 4 weeks of dietary supplementation, CoQ10 phytosome supplementation correlates with more significant improvement in asthenia compared placebo ($p<0.05$). At 8-week follow-up, patients undergoing CoQ10 dietary supplementation experienced significant improvements in asthenia (-30.0±20.0%), HGs (+29.8±3.6%), 1-min STS repetitions (+36.4±3.9%), and 2MST (11.1±1.8%) ($p<0.05$ versus baseline and versus placebo). Chronic dietary supplementation with CoQ10 phytosome is able to effectively improve physical performance in older adults with statin-associated asthenia.

## SEX X TIME INTERACTIONS IN LIPOPROTEIN(A) AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL RESPONSE TO EVOLOCUMAB

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The aim of this study was to evaluate whether there were significant sex x time interactions in lipoprotein(a) (Lp(a)) and low-density lipoprotein cholesterol (LDL-C) response to treatment with the Proprotein Convertase Subtilisin/Kexin type 9 inhibitor (PCSK9i) Evolocumab, in a real-life clinical setting. For this purpose, we pooled data from 176 consecutive patients (Men: 93; Women: 83) clinically evaluated at baseline and every 6 months after starting treatment with Evolocumab. Individuals who had been on iPCSK9 for less than 30 months and noncompliant patients were excluded from the analysis. Over the time, absolute values of Lp(a) plasma concentrations significantly decreased in the entire cohort (P-value <0.001), and in men (P-value <0.001) and women (P-value=0.002). However, there were no sex-related significant differences. Absolute plasma concentrations of LDL-C significantly decreased over the time in the entire cohort (P-value <0.001), and in men (P-value <0.001) and women (P-value <0.001). LDL-C concentrations remained significantly higher in women than men. The sex x time interaction was statistically significant in LDL-C (all P-values <0.05), while absolute changes in Lp(a) were influenced neither by sex nor by time (all P-value >0.05).Our data reinforce presence of differences in response to treatment to PCSK9i between men and women. However, it is still unknown whether these differences translate into a meaningful difference on long-term cardiovascular risk.

## THE EFFECT OF DIETARY SUPPLEMENTATION WITH PLANT STEROLS ON CHOLESTEROLEMIA IS AFFECTED BY ADHERENCE TO MEDITERRANEAN DIET: INSIGHTS FROM THE DESCO RANDOMIZED CLINICAL STUDY

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The DESCO study was a single center, randomized, double blind, placebo controlled, two-way crossover clinical trial designed to investigate the lipid-lowering effect of a new once-a day liquid dietary supplement containing 2.5 g of plant sterols in a cohort of 50 Italian individuals with polygenic hypercholesterolemia who were warmly suggested to follow a low-fat low-sodium Mediterranean diet for the entire duration of the study. Eligible individuals were enrolled in a run-in period of 2 weeks. Then, participants who qualified for continuation in the study -according to the entry criteria- were randomly allocated (1:1) to a 3-week treatment with either phytosterols or placebo. After a 2-week washout period, enrolled individuals were crossed over to receive the alternative treatment. The study was successfully completed by 49 individuals. Dietary supplementation with phytosterols was associated with significant improvement in plasma levels of total cholesterol (TC= -4.8±8.0%), low-density lipoprotein cholesterol (LDL-C= -6.9±14.7%) and apolipoprotein B-100 (Apo B-100 = -2.9±9.1%) compared to baseline. The changes in TC and LDL-C were also significant compared to placebo, and greater adherence to the Mediterranean diet was significantly associated with greater reductions in LDL-C. Dietary supplementation with phytosterols was very well tolerated and adherence to treatment was high. According to the findings of DESCO, the once-a day liquid dietary supplement we tested is able to quickly and significantly decrease plasma levels of TC, LDL-C and Apo B-100, with a greater effect in individuals following the Mediterranean diet.

## AORTIC TRANSCRIPTOME ANALYSIS REVEALS THE ASSOCIATION BETWEEN ATHEROSCLEROTIC LESIONS AND ALTERED SYMPATHETIC INNERVATION IN GENETICALLY MODIFIED MICE

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**Aim.** Previous reports have suggested an association between the development of atherosclerosis and alterations in the aortic sympathetic nervous system, located in the adventitial layer. In a recent study, an increased adventitial axon density in proximity to advanced atherosclerotic plaques of Apoe-deficient mice was observed.
**Methods.** Eight-week-old C57Bl/6J control mice (Bl/6), ApoE knockout mice (EKO), EKO mice overexpressing human apoA-I (EKO/hA-I) and double ApoE/ApoA1 knockout mice (DKO) mice were fed a standard rodent diet or a Western-type diet for 22 weeks. At the end of the dietary treatment the aortic atherosclerosis was quantified, and a high-throughput sequencing approach was used to analyze the aortic transcriptome.
**Results.** On standard rodent diet no atherosclerosis was detectable in EKO/hA-I aortas and a moderate plaque development was observed in EKO and DKO, whereas Western-type diet increased plasma cholesterol levels, led to atherosclerosis development in EKO/hA-I and worsened that in EKO and DKO. The administration of Western-type diet deeply modified the aortic transcriptome. In the three genetically modified mouse lines, an upregulated expression of genes associated with the immunomodulatory response was observed. This was paralleled by a downregulated expression of genes involved in the activity of the aortic sympathetic nervous system. Functional enrichment analysis indicated that the presence of advanced atherosclerosis was accompanied by reduced neuronal generation, modulation of synapse chemical transmission, and catecholamine biosynthesis.
**Conclusions.** A relationship exists between atherosclerosis, dyslipidemia, and sympathetic neurotransmission. Advanced lesions are associated with reduced transcriptional activity related to sympathetic innervation of the aorta.

## THE SEQUENTIAL USE OF LS MEASUREMENT AND DIRECT BIOMARKERS OF FIBROSIS IS ABLE TO IDENTIFY PATIENTS WITH SEVERE NASH

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**Background and Aims.** The identification of patients with severe non-alcoholic steatohepatitis (NASH), who are at greater risk of progression to cirrhosis, and who most deserve targeted interventions and recruitment in clinical trials, is a major priority. Several non-invasive tests (NITs) have been developed to detect patients at risk of severe NASH and to reduce the use of liver biopsy. We conducted a prospective single-centre study aimed at evaluating the diagnostic performance of different NITs in the identification of biopsy- confirmed severe NASH. Moreover, we explored if the sequential use of NITs was able to improve their diagnostic accuracy.
**Method.** 56 consecutive patients (73.2% men; age 52 [22-69] years) performed liver biopsy for the suspicionof severe NASH, defined as the presence of NASH, NAFLD activity score ≥4 and fibrosis stage ≥F3. Simplewet NITs [aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, platelet count, AST/ALT ratio (AAR) and AST to platelet ratio index (APRI)], complex wet NITs, [BARD score, Fibrosis-4 index (FIB-4), NAFLD fibrosis score (NFS), Forns Index and Hepamet fibrosis score (HFS)], direct biomarkers of fibrosis [collagen IV (CIV), laminin (LM), cholylglycine (CG), hyaluronic acid (HA) and procollagen type III amino-terminal peptide (PIIIP)], measurement of liver stiffness (LS) with Fibroscan® , and AGILE 3+ and AGILE 4 scores, were all evaluated at the time of liver biopsy. The area under the receiver operating characteristic curve (AUROC) for the identification of severe NASH of each single NIT was calculated. Finally, we evaluated the diagnostic accuracy of the sequential use of the NITs yielding the best diagnostic performance at AUROC analysis.
**Results.** Severe NASH was present in 12 (21.4%) patients. Simple wet NITs were not able to significantly detect severe NASH (AUROCs ranging from 0.46, p=0.68 for albumin to 0.67, p=0.08 for GOT, platelet and AAR). Among complex wet NITs, only BARD was able to significantly detect severe NASH (AUROC 0.77, p=0.004), while FIB-4, NFS, Forns Index and HFS were not (AUROCs ranging from 0.45, p=0.71 for Forns Index to 0.65, p=0.12 for NFS). All direct biomarkers of fibrosis significantly detected severe NASH, with PIIIP (AUROC 0.81, p=0.001) and CIV (AUROC 0.80, p=0.002) showing the best diagnostic performance. NITs based on LS with Fibroscan® also significantly detected severe NASH, however AGILE 3+ (AUROC 0.71, p=0.03) and AGILE 4 (AUROC 0.70, p=0.04) scores did not improve the diagnostic performance of LS alone (AUROC 0.76, p=0.007). The sequential use of LS (cut-off 8.5 kPa) and PIIIP (cut- off 22 ng/ml) or CIV (cut-off 16.5 ng/ml) had a diagnostic accuracy of 85.7% and 91.1%, respectively.
**Conclusion.** The use of FIB 4 and NFS in high risk populations for severe NASH has a diagnostic accuracy lower than the sequential use of LS in association with direct fibrosis The sequential use of LS and direct biomarkers of fibrosis, such as PIIIP and CIV, may be a promising approach to non-invasively identify patients with severe NASH.

## GENETIC HETEROGENEITY OF FAMILIAL HYPERCHOLESTEROLEMIA: A CASE REPORT

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**Background.** Familiar Hypercholesterolemia (FH) is an inherited disorder of lipid metabolism characterized by high low-density lipoprotein cholesterol (LDL-C) levels and premature atherosclerotic cardiovascular disease (ASCVD). Mutations in three main genes that are responsible for this autosomal dominant disease (LDLR, APOB and PCSK9 genes) and heterogeneity of phenotype mostly depend on the underlying genotype. In addition, variants in genes causing other dyslipidemias, showing phenotypes overlapping with FH, may also act as phenotype modifiers in patients with FH.
**Methods.** A 36-years-old woman was evaluated for hypercholesterolemia, that was known since childhood. Dyslipidaemia and atherosclerotic-based cardiovascular disease occurred at an early age (acute coronary syndrome in the father at age of 45) in many family members. The highest levels of total and LDL cholesterol (542 mg/dL and 398 mg/dL, respectively) were registered during pregnancy, when the hypolipidemic therapy was discontinued. Laboratory data showed high levels of total cholesterol (327 mg/dL) and LDL-C (255 mg/dL) despite simvastatin plus ezetimibe therapy; triglycerides and high-density lipoprotein cholesterol (HDL-C) were 46.9 mg/dL and 62.4 mg/dL, respectively. The DUTCH score 6 was suggestive of clinically probable FH. Next generation sequencing was carried out on an Ion GeneStudio S5 Plus System using the Ion 540 Chip with a custom panel including 50 candidate genes related to LDL, HDL and triglycerides metabolism.
**Results.** The proband was found to be heterozygous carrier of a previously described pathogenic non-sense mutation in LDLR with null LDL-R activity (c.1257C>G p.Tyr419Ter). Interestingly, another mutation was identified on lisosomal acid lipase (LIPA) gene (c.929G>A p.Trp310Ter) in heterozygosis. This variant has been previously identified in homozygosity in two newborns of Sicilian origin with Wolman's disease and in heterozygosity in a subject belonging to the same family (unpublished data). In addition, the determination of Polygenic Risk Score was performed (PRS: 0.652).
**Conclusion.** We report a case of heterozygous familial hypercholesterolemia (HeFH) carrying a composite genotype. The presence of a variant in modifier gene such LIPA in HeFH may further exacerbate the phenotype, partially justifying the variability among patients. Further studies are needed to evaluate how heterozygous variant c.929G>A p.Trp310Ter on the LIPA gene may modulate the lipid and cardiovascular phenotype of FH patients.

## HETEROGENEITY OF THE GENETIC BACKGROUND OF FAMILIAL HYPERCHOLESTEROLAEMIA IN TWO POPULATIONS FROM ITALY AND RUSSIA

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Considering the heterogeneous phenotypic expression of Familial Hypercholesterolemia (FH) based on differences in population genetic background, we aimed to evaluate the genetic profile of FH individuals from Italy and Russia, taking into account the importance of analysing genotype-phenotype manifestations. In this study we included 144 Italian FH patients from one centre of the LIPIGEN study and 79 Russian FH patients from one centre of the Russian Lipid Clinics Network. We collected demographic and clinical characteristics at baseline, and genetic test results. Patients were divided in: positive to genetic test (with one causative mutation), inconclusive (with only variants of uncertain clinical significance [VUS]), and negative (with likely benign/benign variants, heterozygous variants in LDLRAP1 gene, or without causative mutations).The mean age at baseline was comparable (Italy: 47.5±12.5 years, Russia: 45.1±12.4 years; p=0.15); the Russian cohort presents with higher levels of pre-treatment LDL-cholesterol (pre-LDL-C) compared to the Italian population (324.1±91.6 mg/dL vs 296.0±51.8 mg/dL; p=0.01). The genetic test was positive in 75.7% of the Italian subjects and in 49.4% of the Russian subjects, while the presence of only VUS was detected in 8.3% and 19.0% (p<0.001), respectively. Among positive FH patients, pre-LDL-C levels were higher in the Russian cohort (353.5±111.3 mg/dL vs 302.4±52.2 mg/dL; p=0.008), as well as the percentage of treated patients (53.8% vs 13.8%; p<0.0001) and the prevalence of premature coronary heart disease (12.8% vs 3.7%; p=0.041). Among inconclusive subjects, the Russian cohort was about 12 years younger compared to Italians (42.7±12.1 years vs 54.5±10.5 years; p=0.013), while the mean pre-LDL-C levels were similar (299.5±68.1 mg/dL vs 299.0±46.4 mg/dL; p=0.982). Among pathogenic/likely pathogenic variants and VUS, only 5% and 4% was shared between the two cohorts, respectively. Our results highlighted a high variability in the genetic background of patients diagnosed with FH from two different countries, which deserves further investigation.

## STEROL PROFILE ANALYSIS IN AN UMBRIAN FAMILY WITH SITOSTEROLEMIA

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**Background.** Sitosterolemia, also known as phytosterolemia, is a rare autosomal recessive disorder caused by mutations in the ATP-binding cassette subfamily G member 5 (ABCG5) or member 8 (ABCG8) genes. This leads to abnormal functions of the transporter sterolin-1 protein encoded by ABCG5 and sterolin-2 protein encoded by ABCG8, respectively, which can hinder the formation of stable ABCG5/G8 heterodimers, decreasing the ability of intestinal counter-transport of sterols. As a result, phytosterols in plasma and tissue are significantly increased, leading to early onset atherosclerosis-related diseases and xanthelasma of tendons and skin.
**Case descriptions.** The study subjects were two 13-old and 15-old brothers and their parents who had sitosterolemia. All subjects including grandparents (the two maternal and paternal grandmother) were genetically characterized. To confirm sitosterolemia diagnosis, we analyzed plasma sterols by gas chromatography in all subjects at baseline and after three months of Ezetimibe treatment in the two brothers and their parents. At baseline, the two brothers showed normal cholesterolemia and very high levels of phytosterols (63 mg/dL and 55 mg/dL; reference values <1.22 mg/dL). Whereas the two parents had hypercholesterolemia with a slight increase in plasma phytosterols (1.85 mg/dL and 3.21 mg/dL). Moreover, the three grandparents showed hypercholesterolemia together with borderline levels of plasma phytosterols. After Ezetimibe treatment, we observed a reduction (-13% and -34%) of plasma phytosterols in the two brothers and normalization of cholesterol and phytosterols levels in the parents.
**Conclusion.** Sitosterolemia could be effectively controlled after dietary control and oral lipid-lowering therapy with Ezetimibe. Although considered an autosomal recessive disorder, this case report, in agreement with the recent literature, have shown that a heterozygous variant in ABCG5 or ABCG8 genes can also cause mild symptoms.

## LIPOPROTEIN(A) DISTRIBUTION AMONG SUBJECTS WITH GENETIC DYSLIPIDEMIAS: DATA FROM A SINGLE CENTER RETROSPECTIVE STUDY

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**Background.** Lipoprotein(a)[Lp(a)] is a well recognized risk factor for atherosclerotic cardiovascular disease (ASCVD). The interactions between the genetic contribution to Lp(a) plasma levels and the genetic background of dyslipidemias that extremely raise or lower the apoB levels (e.g. familial hypercholesterolemia, FH and familial hypobetalipoproteinemia, FHBL1) is not fully understood. In addition few evidences are available on the distribution of Lp(a) levels among subjects at different cardiovascular (CV) risk (mild, moderate, high and very high).
**Aims and Methods.** The aim of this study was to investigate the distribution of Lp(a) plasma levels in subjects with high and low LDL-C levels (FH and FHBL1) and in a free-living population. The study population included 212 genetically characterized FH subjects, 144 with clinical FH (mutation negative - FHneg), 52 FHBL and 797 free-living subjects. Clinical features and CV risk were also evaluated.
**Results.** Lp(a) levels were significantly higher in FH subjects (both FH and FHneg) (median 12.46 mg/dl and 14.0 mg/dl, respectively) compared to FHBL and controls (7.68 mg/dl and 7.18 mg/dl, respectively). More, Lp(a) levels were similar in FH subjects carrying LDLR defective mutations and in those with LDLR null mutations. Subjects at high and very high CV risk exhibited significant higher Lp(a) levels (median 10.68 mg/dl and 9.20 mg/dl, respectively) compared to low and moderate CV risk (median 5.72 mg/dl and 7.80 mg/dl, respectively) ($p<0.0008$).
**Conclusions.** We have evaluated the distribution of Lp(a) plasma levels in subjects with genetic dyslipidemias and at different CV risk. The FH but not the FHBL1 genetic status interacts with the Lp(a) levels. Subjects at high and very high CV risk exhibited significant higher Lp(a) levels compared to low and moderate CV risk. Furthermore, the identification of subjects with Lp(a) >50 mg/dl may allow to perform a reclassification of CV risk. Combined evaluation of Lp(a) levels in subjects with other traditional risk factors could identify high-risk individuals who may benefit from early aggressive treatments to avoid premature CV events.

## LONG-TERM EFFICACY AND SAFETY OF LOMITAPIDE IN PATIENTS WITH FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS): DATA FROM THE LOCHNES STUDY

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**Background.** Familial chylomicronemia syndrome (FCS) is a rare, severe, monogenic, recessive disorder mainly characterized by very high TG levels and high risk of acute and/or recurrent pancreatitis. Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor approved for the treatment of homozygous familial hypercholesterolaemia. The open-label, single-arm 'LOCHNES' study of lomitapide in adult patients genetically confirmed FCS with a history of pancreatitis (EudraCT 2018-002911-80), showed that lomitapide is effective and well tolerated.
**Methods.** Fourteen FCS patients, previously enrolled in the 'Lochnes' study, were admitted to the Lomitapide Expanded Access Program, 2 months after the study termination and were evaluated every three months over a 2-years follow-up (median 28 months). Each patient continued lomitapide at the maximum tolerated dose as determined during the trial. Lipid profile, liver function tests, fatty liver and hepatic stiffness were evaluated.
**Results.** At the beginning of the follow-up, after 2 months discontinuation of study drug, median TG levels were 1899.5 mg/dL (237-4398 mg/dL). Median fasting TGs at the last observation were 383 mg/dL (47-1678 mg/dL; 76.9% reduction); 9 patients achieved TGs ≤750mg/dL. Adverse events were mild-to-moderate and mainly related to gastrointestinal tolerability (n=11). Over the follow up period 2 patients experienced an acute pancreatitis episode. Liver function tests ≥3x ULN were recorded in 2 subjects. Hepatic fat increased in three patients while median hepatic stiffness remained normal.
**Conclusions.** Lomitapide is effective and well tolerated in reducing TGs in FCS patients with a history of pancreatitis over a 2-years follow-up.

## INCLISIRAN, NEW FRONTIER IN HYPOLIPIDEMIC THERAPY: REAL-WORLD DATA

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**Background.** In October 2023 AIFA approved a new molecule targeting PCSK9, Inclisiran, a siRNA which selectively target hepatocytes where it promotes cleavage of intracellular PCSK9 mRNA, with an advantage versus Evolocumab and Alirocumab, PCSK9-inhibitors (PCSK)-i), relative to the smallest number of administrations. While this drug has shown efficacy and safety in randomized controlled trial, its applicability in real-world clinical settings remains to be elucidated. Therefore, this analysis aims to assess the early effects of this drug in a tertiary centre lipid and cardiovascular risk clinic.
**Population and Methods.** We performed a retrospective analysis of the first 50 patients who received a single dose of Inclisiran at our clinic between 1 January 2023 and 1 July 2023. Data were collected using electronic healthcare records. The lipid profile was assessed before starting the treatment and at 3 months follow-up. Data on adverse events were also recorded.
**Results.** Our population consisted of 50 patients: 16 (32%) female, mean age of 59,24±13 years, 12 (54,5%) with heterozygous familial hypercholesterolemia, 16 (32%) at high risk and 34 (68%) at very hight risk. 36 (72%) patients, re-evaluated at 3 months follow-up, show a mean baseline LDL-C reduction from 130,6±62 mg/dL to 81,1±48 mg/dL by 24,7±49%. Notably in patients not previously treated with others PCSK9-i (n=26), eg. Evolocumab and alirocumab, the median LDL-C relative reduction was 43.8±25%, with a wide range of responses ranging from 8,3% to 87,4%, while in patients who switched from others PCSK9-i to Inclisiran (n=10) we observed a relative increase of 19.6±67%. 11 (30,6%) patients achieved a 50% or greater reduction in LDL-C from baseline; 14 (37,8%) patients reached ESC guidelines LDL-C target: 2 at high-risk patients (LDL-C target <70 mg/dL) and 12 at very high risk patients (LDL-C target <55 mg/dl). Predictably, we could observe a significantly higher probability of reaching LDL-C target at 3 months in patients on triple therapy (p<0.001), showing the role of background lipid lowering therapies (LLTs). Adverse events were recorded in two patients (5,6%): one patient complained headache and dyspepsia, while the other patient reported palpitations and asthenia, resulting in the discontinuation of the therapy.
**Conclusions.** In naïve patients Inclisiran shown an efficacy such as that reported in trials, with good security profile. As might be expected, given the 10% lower efficacy of Inclisiran compared to the other PCSK9-i, patients who switched from Evolocumab or Alirocumab to Inclisiran showed an increase of baseline LDL-C levels, which however remained close to the recommended target. We are aware that Inclisiran has been shown to reach its peak of efficacy after the second administration at 3 months. Thus, further studies on larger samples providing data after the second administration will be needed. At last, despite the effectiveness of these new therapeutic options, background LTTs therapy keep his relevance in order to achieve the LDL-C target.

## MONOGENIC/POLYGENIC HYPERCHOLESTEROLEMIA: DATA FROM FLORENTINE REFERRAL CENTER

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**Background.** As many familial hypercholesterolemia (FH) subjects (about 60%) did not demonstrate functional mutations in major candidate genes (LDLR, APOB, PCSK9, LDLRAP1), we assessed FH patients genetic profile by high-throughput sequencing (HTS).
**Methods.** We analysed 114 FH patients [adults with possible/probable/definite FH according to Dutch Lipid Clinic Network Score (DLCN)]. Targeted HTS (57 genes involved in lipid metabolism, supposed to be involved in dyslipidaemia, pharmacogenetics of statins, related to FH polygenic forms, HDL and triglycerides related diseases) was assessed by Illumina technology.
**Results.** Among 114 patients, 42 carried a rare variant in LDLR gene, whereas 72 patients were LDLR-negative. Talmud score evaluation (Talmud 2013) showed a higher median value in patients LDLR-negative, with respect to LDLR-positive [median(IQR): 1.02(0.93-1.08) vs 0.95(0.85-1.06), p=0.081]. Fourteen out of 42 LDLR-positive patients also carried uncertain significance mutations in other possible FH candidate genes. In patients without LDLR mutations, at least 2 rare variants were identified in 35 patients (49%), and at least 3 rare variants were identified in 27 patients (37%). In these patients, a total of 131 rare variants with uncertain significance have been identified in 44 different genes (APOB, PCSK9, LDLRAP1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, CELSR2, CETP, CREB3L3, DAB2, GCKR, GHR, HFE, ITIH4, LCAT, LIPC, LIPI, LMF1, LPA, LPL, LRP1, MTTP, NPC1, NYNRIN, PON1, PP1R17, SCARB1, SLCO1B1, SLC12A4, SREBF1, SREBF2, SLC22A1, EPHX2, GPD1, OSBPL5, STAP1, ABCA1, DGAT1, INSIG2, NPC1L1, APOA5). Among FH patients, 31 were younger than 18 yrs. Among adults, LDL-cholesterol levels were comparable between LDLR-positive and LDLR-negative group, whereas in younger subjects significantly higher LDL-cholesterol levels were observed among LDLR-positive. As concerns DLCN score, performed in adult population, significantly higher values in subjects carrying LDLR mutation were found.
**Conclusions.** Present data support the involvement of multiple loci beyond LDLR gene in the modulation of lipid profile, as well as cardiovascular risk. Expansion of genetic analysis might allow a better comprehension of the role of further major/modifier genes, as well as of accumulation of common small- effect LDL-C raising alleles in determining LDL-C levels and cardiovascular events.

## VALIDATION OF A NEW WEB-BASED APPLICATION (www.humtelemed.it) TO MAKE EASY AND FAST SCORE2-BASED INDIVIDUAL CARDIOVASCULAR RISK ASSESSMENT

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**Background.** Individual cardiovascular risk (CVR) assessment is based on the SCORE2/SCORE2-OP model endorsed by the 2021 ESC Guidelines on Cardiovascular Disease Prevention using risk charts that, in real-life clinical practice, are very rarely used. We developed a free-to use web application (www.humtelemed.it) based on EAS 2019 guidelines updated with 2021 SCORE2/SCORE2-OP model to simplify CVR assessment for non-expert users or busy physicians. In this study, we assessed the concordance between the charts and the web-app.
**Methods.** A cross-sectional study on 1306 consecutive patients referred to our centre to diagnose and manage hypertension and/or dyslipidemia. Individual CVR stratification (low-moderate, high and very-high) was assessed for each patient using the 2021 ESC Guidelines visual risk charts and the web app (www.humtelemed.it) by two double-blind operators. The marginal homogeneity test was used to evaluate the concordance between the two methods.
**Results.** Mean age was 60±11 years, with male prevalence (53%). Seventy-three percent of patients was in primary prevention. With the SCORE2 charts, patients were classified as 19.5% (255), 35.6% (465), and 44.9% (586) into low-moderate, high and very-high risk, respectively. Our web app classified 18.7% (244), 37.2% (486), and 44.1% (576) patients into low-moderate, high and very-high risk, respectively. According to the marginal homogeneity test, the individual CVR assessed using the web app (www.humtelemed.it) was not statistically different from the CVR using the SCORE2 charts (p=0.907).
**Conclusion.** There is a small, non-statistically significant discrepancy between the two methods, potentially also due to the non-infallibility of manual CVR evaluation method. Our web-based application was specifically developed to help occasional users and busy clinicians to assess individual CVR and may represent a free-to-use, reliable, simple, time-sparing and widely available alternative to the manual CVR evaluation using SCORE2 charts.

## MOLECULAR CHARACTERIZATION OF PATIENTS WITH AND WITHOUT CORONARY ARTERY DISEASE WITH "EXTREME" LEVELS OF HDL-C

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**Introduction.** Observational studies have highlighted the opposite association between LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) and the risk of developing coronary artery disease (CAD). Results of all large scale-randomized trials have demonstrated the causal relationship of LDL-C, while mendelian randomization studies on HDL-C are inconsistent and ambiguous. Targeted Next Generation Sequencing (NGS) analysis in recent years have been developed to study the coding regions and intron/exon boundaries of genes affecting the main pathways of HDL-C synthesis and metabolism in patients with hypo and hyper-alphalipoproteinemia.
**Material and Methods.** The study sample includes patients enrolled in the Verona Heart Study (VHS) with angiographically documented CAD (positive CAD) and subjects without CAD who underwent coronary angiography for other clinical indication (negative CAD). We selected patients with extreme HDL-C phenotype (<2° percentile - 29 mg/dl and >98° percentile - 99 mg/dl). For genetic analysis, a large-scale targeted sequencing analysis was performed on Ion Torrent technology. We selected, for each patient, variants in candidate genes known to be associated to regulate HDL levels.
**Results and Conclusion.** Among subjects with high HDL-C phenotype (>98° percentile - 99 mg/dl) were available samples for 5 patients (5 negative CAD). No pathogenic variants in candidate genes were identified. Among subjects with low HDL-C (<2° percentile - 29 mg/dl) were available samples of 38 patients (31 positive CAD and 7 negative CAD). In this patients we identified an ABCA1 gene mutation c.5398A>C (p.Asn1800His) in heterozygosity in one positive CAD patient. Two variants in ABCA1 (c.103A>G, p.Ile35Val) and CUBN (c.10759G>A, p.Gly3587Arg) of uncertain clinical significance were identified in heterozygosity in one positive CAD patient. Another one (positive CAD) was found to be carrier of the ABCA1 variant c. 2328G>C, p.Lys776Asn in heterozygosity. In conclusion, in most cases the phenotype cannot be correlated to the presence of variants in the candidate genes.

## EVOLOCUMAB AND ALIROCUMAB: A REAL WORLD PHOTOGRAPHY

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**Background.** There is a growing gap between the ideal guidelines LDL-C targets and the LDL-C levels achieved in clinical practice. Indeed, real world data show that about 80% of (very) high risk patients disregarded guideline recommendations. Therefore, our aim was to provide data of monoclonal antibody ihibitors of PCSK9 (PCSK9i) use in clinical practice investigating the adherence to the latest ESC guideline recommendations, with a focus on the role of background oral lower lipid therapy (LLT) in the probability of target attainment.
**Methods.** Between April 2018 and December 2023, patients evaluated at our center who started PCSK9i therapy (evolocumab 140 mg or alirocumab 150 mg) were included in a prospective registry. The lipid profile was assessed before starting PCSK9i therapy, and during follow-ups that were per-formed every six months, with a median follow-up of 24 (6-48) months. Results are presented for the total population and stratified by patient subgroups: high risk patients with asymptomatic heterozygous familial hypercholesterolemia (LDL-C target <70 mg/dl) and very high risk patients with known atherosclerotic cardiovascular disease or diabetes mellitus with target organ damage or additional major risk factor (LDL-C target <55 mg/dl).
**Results.** Our cohort consisted of 271 patients: 100 (36,9%) women, mean age of 65,1±11,1 years, 60 (22,1%) at high risk and 211 (77.9%) ad very high risk, the majority (75,2%) with a diagnosis of coronary artery disease. At the first 6 month follow up, mean baseline LDL-C values decreased from 144,8±52,7 mg/dL to 59,3±37,4 mg/dL (from 183,8±46,9 to 75,1±37,8 in high risk patients and from 130,9±41,5 to 55,2±36,4 in very high risk patients), with a mean relative reduction of 57.4±26.1%, similar in the two subgroups and between the two class of PCSK9i.At 6 months 57.7% patients reach their LDL-C target according to the latest ESC guidelines: 51.1% patients at high risk (target <70 mg/dl), and 59.4% at very high risk (target <55 mg/dl). The percentage of patients on target did not change significantly in subsequent follow-ups. Background oral hypolipidemic therapy was a predictor of optimal LDL-C control: the presence of at least one lipid lowering drug was associated with significantly lower LDL-C levels (87,1±36,1 mg/dl vs 59,5±8,7, p<0,001) and consequently with a significantly higher probability of reaching the target (59,6% vs 20,6%, p<0,001). Three patients underwent percutaneous coronary revascularization for an acute coronary syndrome (2 and 6 month after starting the treatment). During follow-up, five patients switched to Inclisiran therapy, 2 due to adverse events (flu-like syndrome) and 3 for reduced therapeutic adherence.
**Conclusions.** In conclusion, our analysis confirms that PCSK9i are safe and effective drugs, allowing most patients of our cohort to reach LDL-C target through a 55-60% reduction. Among the predictors of therapeutic failure, the absence of concomitant oral lower lipid therapy played a key role, confirming the value of combination therapy in the management of patients at (very) high cardiovascular risk and the importance of its persistence over time.

## STATIN ASSOCIATED AUTOIMMUNE MYOPATHY IN THE PRESENCE OF AUTOANTIBODIES AGAINST 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-COA) REDUCTASE

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**Introduction.** Statin induced muscle weakness is the most common side effect of statin therapy. Rarely, it occurs in the form of myopathy, that will relieve after discontinuation of statin. In exceptionally rare cases (2-3 of every 100.000 statin users), an autoimmune myopathy may develop, associated with autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (1).
This is an only recently recognized form of rhabdomyolysis, and its best management is still unclear (2). Here we describe a rapidly deteriorating and therapy-resistant case.
**Case report.** A 63 years-old Caucasian male with a history of one-month symmetrical muscle weakness, dysphagia and fever presented to our department in December 2021. On admission laboratory results showed extremely elevated creatine kinase and myoglobin levels (CK 28652 U/L, normal range 39-300, myoglobin 6664 mcg/L, 28-72). The patient was taking atorvastatin from one year for mixed dyslipidaemia, and suffer from autoimmune hypothyroidism.
Common causes of rhabdomyolysis were excluded, as well as infectious diseases. High levels of anti-HMG-CoA reductase antibodies were detected, and muscle biopsy confirmed necrotizing myopathy. Treatment was started with intravenous methylprednisolone (1 g/day) and intravenous immune globulin (IVIG, 2 g/kg). However, muscle weakness progressed, and the patient developed dysphagia. The treatment was then intensified by adding rituximab (2 x 1 g, separated by an interval of 2 weeks) and cyclophosphamide (1.2 g every 4 weeks).
The clinical situation further deteriorated, and the patient developed a severe respiratory distress, that required orotracheal intubation, and then tracheostomy. At 45 days from hospital admission, plasmapheresis over 5 days was finally performed. As expected, anti-HMG-CoA reductase antibody levels and CK levels decreased. Notably, symptoms progressively improved over the following 2 weeks after plasmapheresis, and the patient passed to spontaneous breathing. IVIG were continued and corticosteroids tapered. Two months after admission, the patient was discharged to a rehabilitation hospital, with a maintenance dose of methotrexate, rituximab, and repeated cycles of IVIG over 6 months. Despite several infections (of those COVID19), and an episode of venous thromboembolism, at one and a half year, patient symptoms greatly improved, but he only partially recovered his previous functional status.
**Conclusions.** Statin-associated anti-HMG-CoA reductase myopathy is a rare but severe autoimmune myopathy, with potentially high morbidity and mortality. It must be promptly diagnosed, as it requires rapid and profound immunosuppressive therapy. Plasmapheresis might help to interrupt pathogenic events, and to improve symptoms during the acute phase of the disease (3). In these patients, re-treatment with statins should be avoided. Therefore, the new lipid lowering agents could represent the treatment of choice to reach the therapeutic target.

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## DISCOVERING NEW LDL RECEPTOR MODULATORS: THE ROLE OF CARBAMOYL PHOSPHATE SYNTHASE 1 (CPS1), A MITOCHONDRIAL ENZYME

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**Background and Aim.** LDL receptor (LDLR), chiefly expressed in liver, is the main regulator of LDL plasma levels. The aim of this study is to identify differentially expressed proteins (DEPs) involved in LDLR modulation and/or in chol/TG metabolism.
**Methods.** HuH7 human hepatocarcinoma cell line was incubated with fluorescently labeled LDL (LDL- DyLight550TM) and sorted in two subpopulations naturally expressing high and low LDLR. Western Blotting and qPCR have been used to evaluate proteins and transcripts levels, respectively.
**Results.** The proteomic analysis on HuH7 High and Low pointed out an enrichment in several mitochondrial DEPs. Among them, carbamoyl phosphate synthase 1 (CPS1), that catalyzes the rate limiting step of the urea cycle, resulted significantly upregulated in High cells compared to Low cells. Knocking-down CPS1 in HuH7 cells with a specific 20nM siRNAs pool resulted in a significant decrease in LDLR mRNA levels (-40%, $p<0.05$ vs scramble), thus confirming the positive correlation between LDLR expression and CPS1 expression resulted from the proteomic analysis. Surprisingly, we observed a significant decrease in PCSK9 (-50%, $p<0.05$), HMG-CoA reductase (-90%, $p<0.001$), and SREBP2 (-70%, $p<0.01$) mRNA levels compared to scramble cells.
**Conclusions.** The proteomic analysis shed light on several up- and down-regulated mitochondrial DEPs between HuH7 with High and Low LDLR expression, including CPS1 involved in urea cycle, that resulted significantly upregulated in High cells. CPS1 knock-down leads to a significant decrease in LDLR, PCSK9, HMG-CoA red mRNAs, as well as in SREBP2 transcript, suggesting a modulation via this pathway. Given these results, we are tempting to speculate that mitochondria are essential components of an extramitochondrial function: regulation of LDLR availability on the plasma membrane, thus paving the way to new therapeutic scenarios in cholesterol dysfunctional diseases.

## IN VITRO PHARMACOLOGICAL STUDY OF MONOCLONAL ANTIBODIES AND SMALL-INTERFERING RNA ANTI PCSK9: POTENTIAL CLINICAL PROSPECTIVES

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**Background.** Immediate and aggressive lipid lowering therapies after acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI) is recognized by the ESC/EAS dyslipidemia guidelines, which recommend high-intensity statin therapy within the first 1-4 days of hospitalization.In the same clinical setting, the efficacy of monoclonal antibodies (mAbs) anti PCSK9, evolocumab and alirocumab, is under investigation. A recently discovered means of decreasing PCSK9 levels is the administration of small interfering RNA (siRNA) molecules. A further development of siRNA anti PCSK9 led to the identification of inclisiran, a liver-specific, long-acting, subcutaneously delivered, synthetic siRNA directed against PCSK9 and conjugated to triantennary N-acetylgalactosamine carbohydrates (siRNA-GalNAc). Inclisiran acts through an intracellular mechanism by degrading the mRNA and inhibiting the translation of PCSK9, while evolocumab acts on circulating PCSK9 with a direct and rapid binding. Since circulating PCSK9 is mainly, but not exclusively, from hepatic origin, the liver specific action of inclisiran may explain a non-complete inhibition of PCSK9 (approximately -75%), while evolocumab binds almost total PCSK9 (approximately -97%). In this scenario, appropriate pharmacodynamic and pharmacokinetic characteristics of anti PCSK9 therapies are a prerequisite for an effective pharmacological action.
**Aim.** In this study, we investigated the kinetic of PCSK9 inhibition by siRNA anti PCSK9 and determined the effect of this inhibitor on low-density lipoprotein receptor (LDLR) expression, ApoB and PCSK9 secretion in human hepatocarcinoma cell line Huh7.
**Material and Methods.** Huh7 cells were incubated at different time-points with siRNA anti PCSK9 (siGENOME SMARTpool, Dharmacon), evolocumab (Amgen) or human recombinant PCSK9 (Cayman Chemical). LDLR and PCSK9 expression were determined by western blot analysis. From the conditioned media apoB and PCSK9 were measured by using specific ELISA kits. LDL- DyLightTM550 (Cayman Chemical) uptake were determined by cytofluorimetry.Results. siRNA very efficiently reduced the intracellular levels of PCSK9 after 24 and 48 hours (-60 and -80%, respectively). On the contrary, the determination of extracellular levels of PCSK9 showed a stronger effect after 48 and 72 hours (-60%), while no effect was observed on apoB secretion. Shorter time-course experiments revealed that siRNA reduced intracellular PCSK9 protein expression from 4 to 8 hours post treatment (-30 and -40%, respectively), while no effect is observed at shorter time points. The effect on secreted PCSK9 was observed starting from 8 hours (-40%). Surprisingly, the reduction in PCSK9 levels only marginally, and not significantly, reduced the LDL receptor expression. Long-term incubation with evolocumab (48 hours) determined a significant reduction of both intracellular and secreted PCSK9 (-40%), while no effect was observed at 24 hours. Evolocumab (10 µg/mL) itself didn't show a modulation of LDL receptor, while incubation with recombinant human PCSK9 (5 µg/mL) for 24 hours induced a complete degradation of the receptor, effect rescued by the co-incubation with evolocumab. Finally, Huh7 very efficiently uptake recombinant PCSK9 either in the presence or absence of evolocumab. In line with LDL receptor expression profile, recombinant PCSK9 strongly reduced the LDL-DyLight uptake by Huh7, while the combination with evolocumab increased the uptake. Using this approach, a trend of higher LDL-DyLight uptake by Huh7 cells was observed in response to evolocumab on its own.
**Conclusions.** Both siRNA and mAbs anti PCSK9 did not significantly affected the LDL receptor expression most likely due to the very low intracellular and extracellular concentration of PCSK9 in Huh7 cell line. Instead, in the presence of exogenous recombinant PCSK9, LDLR undergoes degradation, this effect is reversed by evolocumab. This data suggest that alternative in vitro models should be used for studying the effect of siRNA anti PCSK9 (i.e. inclisiran), such as hepatic cell line overexpressing PCSK9 or a more sensitive method for LDL uptake determinations.

## NON-ALCOHOLIC FATTY LIVER DISEASE: EFFECT OF LYCOPENE-ENRICHED FUNCTIONAL FOOD

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**Background.** Currently, there is no approved medication for non-alcoholic fatty liver disease management. Preclinical and in vitro studies have shown that lycopene and carotenoids have been associated with improvement in fatty liver. Starting from these evidences, our aim was to evaluate the effects of a functional tomato sauce with a high lycopene content from vine-ripened tomatoes (namely OsteoCol® – Patent n. 102019000000061) as a treatment for adults with fatty liver disease.
**Methods.** A total of 98 participants with liver steatosis were enrolled in a randomized, double-blind, placebo controlled clinical trial. Participants were allocated to OsteoCol® (80 ml/day) or control tomato sauce (80 ml/day) treatment, for 6 weeks. The lycopene content in OsteoCol® was approximately 20% higher than in the control sauce. The primary outcome measure was the change in liver fat content (CAP score). CAP score, by transient elastography, serum glucose, lipids, transaminases, and cytokines were measured at baseline and after intervention.
**Results.** We analyzed the data of 84 participants after 6 weeks of treatment. After adjustment for confounding variables (i.e., lipid-lowering drugs, AST, liver stiffness at baseline, weight change, fatty mass, liver stiffness at follow up), we found a greater CAP score reduction (%) in OsteoCol group rather than control (-11% vs. -2 %, p=0.010; respectively). The CAP score reduction (%) was even greater in men (-15 % vs. -5%; p=0.042), in obese participants (-13% vs. -0.6%; p=0.022), as well in participants with insulin resistance (-9% vs. -0.03%; p=0.021).
**Conclusion.** A novel functional tomato sauce with a high licopene content from vine-ripened tomatoes (OsteoCol®) was safe and effective in reducing liver fat content over 6 weeks in individuals with hepatic steatosis. The study is still ongoing and will continue for another 6 weeks.

## RISK OF ATHEROSCLEROTIC DISEASES AND NAFLD IN HYPERTENSIVE PATIENTS RELATES TO SLEEPING DISORDERS

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**Introduction.** Nonalcoholic Fatty Liver Disease (NAFLD) defines liver conditions caused by cell fat infiltration in subject with no history of alcohol addiction. TYG score is a risk score for NAFLD that, as well as sleep apnoea, might relate to an increased risk of atherosclerotic diseases.
**Aim.** With this study we would to highlight how OSAS and NAFLD relates ASCVD (Atherosclerotic Cardiovascular Disease).
**Methods.** We evaluated 121 caucasian hypertensives with a negative AUDIT score for alcohol addiction. Patients were evaluated by physical examination (BMI, waist circumference) and routine lab tests.Lausanne NoSAS Score, ASCVD Risk Score and TYG (triglycerides × glucose) index were calculated. Patients were divided respectively with and high risk of OSAS in Group H (NoSAS Score ≥8; 82 patients, 47 males, 66.72±8.67 y) and low risk in Group L (NoSAS Score <8; 48 patients, 14 males, 60.72±13.43 y).
**Results.** Group H presented an increased BMI and waist circumference ($p<0.0001$ both). An increased rate of diabetes was also present (group H 21.95% vs Group L 2.56%, $p=0.006$). HDL was significantly decreased in group H ($p=0.027$). No difference there was in blood glucose, triglycerides, total and LDL cholesterol and, in particular, SBP, DBP and HR were similar. On the contrary, a significant increase occurred in ASCVD risk in group H (Group L 9.04±10.70% vs Group H 15.65±10.49%, $p=0.008$). A significant direct correlation highlighted between ASCVD and both TYG ($r=0.274$; $P<0.005$) and NoSAS ($r=0.403$; $p<0.005$).
**Conclusion.** All patients with an increased OSAS risk have a tight correlation with a high risk of NAFLD and, in particular with an enhanced ASCVD score. This result shows the close correlation between sleeping disorders and NAFLD which might be involved to the onset of ASCVD. Further studies will enhance, then, the primary and secondary prevention of cardiovascular events, including factors still considered extracardiovascular.

## HIGH DIETARY INFLAMMATORY INDEX ASSOCIATES WITH INFLAMMATORY PROTEINS IN PLASMA

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**Background and Aim.** Unhealthy dietary habits and highly caloric foods trigger metabolic alterations and promote the development of chronic inflammatory conditions such as obesity, insulin resistance, type 2 diabetes, and cardiovascular diseases. Describing an inflammatory effect of diet is difficult to pursue, owing lacks of standardized quali-quantitative dietary assessments. The Dietary Inflammatory Index (DII) has been proposed as an estimator of the pro- or anti-inflammatory effect of nutrients and higher DII values, which indicate an increased intake of nutrients with pro-inflammatory effects, relate to an increased risk of metabolic and cardiovascular diseases and we here assessed whether they reflect biologically relevant plasmatic variations of inflammatory proteins.
**Methods.** In this cross-sectional study, seven days' dietary records from 663 subjects in primary prevention for cardiovascular diseases were analyzed to derive the intake of nutrients, foods and to calculate DII. To associate DII with the Normalized Protein eXpression (NPX), an index of abundance, of a targeted panel of 368 inflammatory biomarkers (OlinkTM) measured in the plasma, we divided the population by the median value of DII (1.60 (0.83-2.30)).
**Results.** 332 subjects with estimated DII over the median value compared with subjects with estimated dietary DII below the median value (N=331) reported a higher intake of saturated fats and lower intakes of poly-unsaturated fats, including omega-3 and omega-6 fats. The NPX of 61 proteins was increased in the plasma of subjects with DII>median vs subjects with DII<median. By contrast, in the latter group, we identified only 3 proteins with increased NPX. Vice versa 23, out of these 64 proteins, accurately identified subjects with DII>median (Area Under the Curve=0.601 (0.519-0.668), $p=0.035$).
**Conclusion.** This large-scale proteomic study supports that higher DII reflects changes in the plasmatic abundance of inflammatory proteins. Larger studies are warranted to validate our findings.

# VLDL CHOLESTEROL ASSOCIATES WITH HIGHER PLASMATIC EXPRESSION OF INFLAMMATORY PROTEINS AND ATHEROSCLEROTIC PATHWAYS COMPARED TO LDL CHOLESTEROL

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**Background and Aim.** High cholesterol in Low Density Lipoproteins (LDL-C) is the key target of current pharmacological treatments aimed at reducing atherosclerotic cardiovascular disease (ACVD) risk. Increased cholesterol in very low-density lipoproteins ("VLDL-C") is an independent predictor of ACVD. VLDL-C was previously associated with markers of inflammation (for instance C-reactive protein). We now tested the relationship between either VLDL-C or LDL-C with a large spectrum of inflammatory proteins in plasma collected from subjects at different ACVD risk.
**Methods.** We measured 276 proteins (OlinkTM) in plasma from a primary ACVD risk prevention cohort ("PLIC" in Milan; n=656 (8.2% on statins)) and a secondary ACVD risk prevention cohort (the Second Manifestations of ARTerial disease, "SMART", the Netherlands, n=630 (50.8% on statins)). Cohorts were divided into three groups for VLDL-C ("Normal" VLDL-C<15 mg/dL, "High" VLDL-C 15-30 mg/dL, "Very high" VLDL-C >30 mg/dL) and LDL-C ("Normal" LDL-C <115 mg/dL, "High" LDL-C 115-155 mg/dL, "Very high" LDL-C>155 mg/dL). The expression (Normalized Protein eXpression, NPX) of each protein was compared among these groups by artificial intelligence and the performance to discriminate subjects with higher VLDL-C or LDL-C was evaluated comparing the Areas Under the Curve (AUCs) of the Receiver Operating Characteristics curve (ROC) considering proteomics on top of ACVD risk factors ("CVRFs": age, body mass index, systolic blood pressure, glycemia, therapies), versus the AUC of the ROCs with CVRFs alone.
**Results.** The number of plasma proteins differentially expressed increased as a function of higher VLDL-C in PLIC, as the NPXs of 84 were higher in "High" and the NPXs of 136 were higher in "Very high" vs "Normal" VLDL-C respectively. A similar trend was found in SMART, where the NPXs of 30 proteins were higher in "High" and the NPXs of 64 were higher in "Very high" vs "Normal" VLDL-C respectively. 26 proteins were shared between the two populations and recapitulated key atherosclerotic pathways (including chemotaxis of immune cells). The relationship between LDL-C was less marked; in PLIC, 14 proteins were more expressed in "High" and 33 in "Very high" vs "Normal" LDL-C respectively, while in SMART, the NPXs of 11 proteins were higher in "High" and the NPXs of 36 were higher in "Very high" vs "Normal" LDL-C respectively. Only 4 proteins were shared between high and very high LDL-C in the two populations. Finally, none of the proteins were shared between the groups of high/very high VLDL-C and high/very high LDL-C in the two cohorts. Canonical CVRFs alone slightly improved the ability to identify subjects with increased VLDL-C both in PLIC and SMART (AUCs between 0.6 on average), but adding plasma proteomics markedly improved the performance to identify subjects with "High" VLDL-C, in PLIC (AUC=0.767 (0.709-0.837)) and in SMART (AUC=0.781 (0.681-0.873)), and with "Very high" VLDL-C (AUC=0.950 (0.899-0.976) in PLIC, and AUC=0.938 (0.894-0.971) in SMART).The ROC of plasma proteomics with CVRFs was also superior to the ROC of the CVRFs alone to identify subjects with "High" and "Very high" LDL-C, but, as compared to the ROCs that discriminated subjects with "High" and "Very-high" VLDL-C, the AUCs were attenuated in both cohort (for "High" LDL-C: AUC=0.665 (0.558-0.774) in PLIC and AUC=0.775 (0.704-0.842) in SMART; for "Very high" LDL-C: AUC =0.776 (0.694-0.854) in PLIC and AUC=0.882 (0.825-0.931) in SMART).
**Conclusion.** High VLDL-C associates with a higher number of differentially expressed plasma proteins versus high LDL-C and none of the proteins were in common. Our data do not underestimate the value of LDL-C in ASCVD but reinforce the concept that VLDL may also promote different atherosclerotic pathways involved in determining ACVD.

## STRUCTURE AND TRAFFICKING OF PCSK9 IN LDL-C BINDING

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**Introduction.** Circulating PCSK9 is known to interact with the LDL-R thus promoting its degradation and blunting the uptake of LDL from the circulation. In this context, anti-PCSK9 mAbs and siRNAs are approved for the treatment of hypercholesterolemia. Previous studies have demonstrated that a significant proportion of circulating PCSK9 associates to LDL. The purpose of our research is to better understand the basis of the PCSK9-lipoprotein interaction and whether it has a physiological impact.
**Methods.** A three-layered iodixanol gradient was used to isolate lipoproteins fractions from patients' plasma before and after treatments. Lipoproteins components were studied by spectrophotometric, lipidomic and proteomic approaches.
**Results.** The LDL-C levels decreased from 94±41 to 41±17 mg/dL after siRNA treatment and from 125±52 to 62±40 mg/dL after mAbs therapy. Circulating PCSK9 decreased 70-80% after siRNA, while plasma PCSK9 levels increased 10-fold after mAbs (n=18 and 30 respectively; $p<0.05$). Independent of the therapy, PCSK9-bound to LDL was on average 10% (n=30; $p<0.01$). Immunoblotting analysis demonstrated that PCSK9 circulates also as acetylated protein and is bound to LDL. By lipidomic analysis, PCSK9 associates to a LDL subfraction that has a lower density than average LDL, due to its composition: 17.16% proteins, 28.62% phospholipids, 14.41% triglycerides and 34.81% cholesterol. By LC-MS, this subfraction showed a higher amount of ApoE, ApoCI, ApoCII, ApoCIII than LDL fraction (89±10 and 11±1; 64±8 and 6±1; 76±10 and 15±2; 53±5 and 6±1 attomoles/femtomoles ApoB respectively; $p<0.05$).
**Conclusions.** Our study identified a LDL subfraction more buoyant than the classical LDL (IDL-like lipoprotein) involved in PCSK9 binding. Although the therapies significantly modify the total amount of circulating LDL and PCSK9, the percent of PCSK9-bound to LDL remains constant. The acetylated form of PCSK9 seems to be involved in the LDL binding; whether the rate of acetylation contributes to the total PCSK9 bound remains to be addressed.

## IN VITRO AND IN VIVO STUDY ON NEW DERIVATIVES OF POTENT PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN 9 INHIBITORS

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This study aims to determine the safety and efficacy in vitro and in vivo of new analogues of a recently identified family of Proprotein Convertase subtilisin/kexin 9 inhibitors (MR compounds). It is well established that PCSK9 regulates the degradation of hepatic LDL receptors, thus controlling plasma cholesterol levels. Currently, the only approved PCSK9 inhibitors are biotechnology drugs, such as Inclisiran (a siRNA), Alirocumab, and Evolocumab (monoclonal antibodies), which require high production costs and need to be administered subcutaneously by specifically trained personnel. Based on these premises, potentially orally bioavailable small-molecules may be a valuable addition to existing treatments. N=30 MR compounds were tested preliminary in vitro at increasing concentrations in human hepatocyte cells (HepG2) to evaluate cytotoxicity (MTT assay) and their efficacy in inhibiting PCSK9 secretion (ELISA assay). After preliminary in vitro screening, three compounds (MR-3, MR-532, MR-533) were selected to test toxicity and bioavailability in vivo. These were subcutaneously administered in wild-type mice (C57BL/6J) at a dose of 40 mg/kg for 7 days. To evaluate tolerability and macroscopic toxicity during the experiment the body weight and the locomotion of animals were monitored. After the sacrifice, hepatic toxicity (histological analysis) and biodistribution (LC-MS/MS) were evaluated.MR-3, MR-532 and MR-533 resulted not cytotoxic (IC50=32.4 uM; 35.7 uM; >50 uM, respectively) and showed great efficacy in PCSK9 inhibition in HepG2 cells (IC50=1.7 uM; 5.7 uM; 6.1 uM, respectively). All selected compounds were safe in vivo, showing no signs of hepatic toxicity. MR-3 and MR-532 were detected in plasma (437,22 ng/ml; 220,5 ng/ml). In conclusion, the three compounds tested in vivo proved to be safe and two of them show plasma bioavailability showing promise as PCSK9 inhibitors. Further studies in vivo will be necessary to evaluate whether these compounds are effective in inhibiting PCSK9 in hypercholesterolemic animal models.

## A NOVEL ABCA1 MUTATION IN THE TANGIER DISEASE ASSOCIATED WITH NEURODEGENERATIVE CONDITION

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We report a novel ATP Binding Cassette A1 (ABCA1) gene truncation due to a homozygous mutation (intron 12, c.1510-1G>C) in a 68-year-old male proband with low levels of HDL cholesterol, premature coronary heart disease (CHD), orange tonsils, thrombocytopenia and atypical neurodegenerative disease. The patient came to our attention for a deficit of apolipoprotein A1, known since he was 30 years old. He is a former smoker (20 cigarettes/die for several years; discontinued in 2010) and had a hypertensive ischemic heart disease (Coronary Artery Bypass Grafting in 1989 and two percutaneous transluminal coronary angioplasties with stent in 2006 and 2013). He has a negative family history of cardiovascular events, his diet is controlled and he walks every day from 10 to 60 minutes. His plasma lipid concentration were: total cholesterol 64 mg/dl, HDL cholesterol 6, LDL cholesterol 40 mg/dl, APOA1 undetectable, APOB 68, Lp(a) 3.8 and triglycerides 215. The liver, kidney, thyroid function test and creatine kinase levels were normal. During the interview, in the presence of the partner, the presence of expression aphasia appears evident. In 2019 he began to show mood alterations and lack of affectivity which got worse over the following months. MMSE was certified at 19. MRI of the brain showed: diffuse atrophy. Neurologists concluded with an atypical neurodegenerative form. In February 2021 a PET revealed: frank hypocaptation left fronto-temporo-parietal and right frontal, tracer for amyloid positive result.

**Methods.** Library preparation and sequencing by synthesis on the Illumina platform (kit TruSight One Expanded o TruSight Cardio and sequencing on NEXTSEQ550). The bioinformatic analysis involves the use of BWA Aligner or DRAGEN Enrichment software. The sequences are aligned to the human reference genome GRCh37 which allows the calling of single nucleotide and structural variants. Illumina Variant Interpreter software or TGEX Software was used for variant analysis. For the selection of genes associated with the clinical indication, the OMIM (Online Mendelian Inheritance in Man), PanelApp and Orphanet databases were used. Only variants with read depth >20x and adequate quality parameters were considered Variants are annotated according to the HGVS nomenclature and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Analysis of the impact of the variants on the encoded protein was performed with the following software: SIFT, PolyPhen2, GERP, Phylo P, PhastCons, Mutation Taster, Mutation Assessor, DANN, Align GVGD and Grantham Score. For variants that impact the splicing mechanism, the Alamut software is used. The genetic analysis allowed us to find the variant c.1510-1G>C in intron 12 of the ABCA1 gene, with probable consequences for the splicing of the protein. This variant, present in homozygosity in the patient, is classified as having uncertain significance by the analysis software and as likely pathogenetic by Varsome. The variant is not reported in the medical literature. ABCA1 has a significant impact on amyloid beta deposition (Aβ deposition) and clearance. This is due to the efflux of apolipoprotein E (ApoE) mediated by ABCA1 which is altered in Tangier disease (1). Apo E is the major genetic risk factor for late onset of Alzheimer disease (AD) so the regulation of apo E level may be involved in AD pathogenesis (1), as confirmed by many studies (2-5).

**Conclusions.** This novel homozygous ABCA1 mutation is associated with markedly decreased levels of HDL-C, plasma apoA-I as well as decreased total cellular cholesterol efflux and may play a role in the neurodegenerative condition which affect our proband. Its role in neurodegenerative disorders must be better understood.

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## EFFECTS OF ANDROGEN THERAPY ON AN IN VITRO MODEL OF FATTY LIVER DISEASE

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**Introduction.** The increasing prevalence of non-alcoholic fatty liver disease (NAFLD) and its relationships with many endocrine diseases represent real challenges today. Many liver diseases are accompanied by disorders in lipid metabolism. Hypogonadism, a major problem in sexual dysfunction, is strongly associated with NAFLD. Preclinical and clinical studies have shown that testosterone replacement therapy improves hepatic steatosis, but the molecular mechanism of this effect remains unknown. Starting from this evidence, our aim was to evaluate the effects of Testosterone (TST) and Dihydrotestosterone (DHT), the active metabolite, on different hepatocyte cultures.
**Methods.** We evaluated the effect of TST and DHT at doses of 10 and 100 nM for 24 hours in rats MCA-Rh7777 and human HepG2 hepatic cells. We evaluated the intracellular lipids and triglycerides content. In addition, to understand the mechanism underlying this reduction, we evaluated the genes involved in the metabolism of lipogenesis, lipolysis and β-oxidation, as well as the pathways involved.
**Results.** In rats MCA-Rh7777, the incubation with TST and DHT reduced intracellular neutral lipid and triglycerides content. These results are confirmed also on human HepG2 cell. In addition, in HepG2 cell line, we shown that TST decreases genes involved in lipogenesis (SREBP-1c and DGAT1: 10 nM and 100nM TST vs CTRL, $p<0.001$ respectively; SREBP-2: 10nM TST vs CTRL, $p=0.01$), and increases gene involved in β-oxidation (PPARα: 100nM TST vs CTRL, $p=0.01$) metabolism. Furthermore, TST incubation increased proteins expression levels of SIRT-1 ($p<0.05$) leading to reduction of protein level of PPARγ ($p=0.04$). Incubation with DHT androgen also decrease genes involved in lipogenesis (SREBP-1c: 10 nM DHT and 100 nM DHT vs CTRL, $p=0.002$ and $p=0.01$, respectively; DGAT1: 100 nM DHT vs CTRL, $p=0.01$; PPARγ: 100 nM DHT vs CTRL, $p=0.004$), and β-oxidation (PPARα: 100 nM vs CTRL, $p=0.02$) metabolism. In addition, DHT incubation increased proteins expression levels of SIRT-1 ($p=0.002$) and reduced of protein level of PPARγ ($p<0.001$).
**Conclusion.** We found, for the first time, that replacement therapy with androgens reduced liver fat accumulation thought the alteration of the expression levels of genes and proteins, such us SIRT-1 and PPARγ, involved in hepatic fat accumulation and removal.

## EVALUATION OF ADHERENCE TO LONG-TERM PHARMACOLOGICAL LIPID-LOWERING THERAPY IN OUTPATIENT PATIENTS

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**Background.** Adherence to lipid-lowering therapy is crucial in reducing cardiovascular risk. Several methods can be used to assess therapeutic adherence. The Morisky scale is a validated one and widely used as a measure of adherence.
**Patients and Methods**. We aimed to assess the adherence to pharmacological therapy in the first 160 patients with dyslipidemia consecutively attending our outpatient clinic dedicated to cardiovascular prevention and already under pharmacological treatment for at least twenty-four months (average 33.4±12.2). The four-item Morisky scale was used (good adherence score <3). Out of the 160 patients who were given the questionnaire, eight did not complete it in full. Among the remaining 152 patients, 64 were females and 88 were males. The mean age was 65.38±13.38 years, and the average number of drugs taken was 2.65±1.76.
**Results.** Among the 152 patients who completed the assessment, eight (5.6%) showed low adherence (score ≥3). The average number of drugs taken by these patients was 3.55±0.8.Discussion. The Morisky scale is validated in assessing forgetfulness in drug intake, intentional changes in dosage and mode of administration, and the difficulty in following a specific therapeutic regimen. In our study, the intake of therapy in a consecutive group of 160 patients with dyslipidemia under treatment with lipid-lowering drugs for a long time showed excellent adherence to the pharmacological therapeutic regimen (94.75%). As expected, in patients with low adherence, a higher number of prescribed drugs were noted compared to the overall average of patients.
**Conclusions.** In an outpatient clinic, dedicated to cardiovascular prevention, in patients on long-term follow-up, excellent adherence to pharmacological lipid-lowering therapy was observed. As expected, the few patients with poor adherence had a higher total number of prescribed drugs compared to the sample average in the study.

## LDL-C REDUCTION AFTER ADDING INCLISIRAN IN HIGH/VERY HIGH CV-RISK PATIENTS WITH OR WITHOUT STATIN-EZETIMIBE INTOLLERANCE

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**Introduction.** In spite of the progressive rise in the therapeutic armamentarium for LDL-C reduction in the last decade, a substantial cardiovascular (CV) risk is still present in many patients. Currently those who do not reach LDL-C targets are generally addressed to a combination of statins+ezetimibe. If this is still insufficient to achieve the desired target, a PCSK9-antagonist or inclisiran (an siRNA for PCSK9) can be added. This novel approach could fill the gap in achieving LDL-C target levels in patients with high/very high CV-risk (1).
**Methods.** Ten subjects with high/very-high CV-risk were considered in this real-word study.after attempting to lower LDL-C with different drugs: six patients were on rosuvastatin at maximal tolerated dose in combination with ezetimibe, one on monacolin-K+ezetimibe and one with fenofibrate. Only two of them were without hypolipidemic-therapy for total intolerance. All were then treated with additional inclisiran by s.c. injection (at days 1-90), five have already received an injection after 270 days.
**Results.** The mean value of LDL-C at baseline was 125.4 mg/dl ±38.6. Percentage LDL-C changes after inclisiran were remarkable, with mean reductions of 42-45%. Three patients achieved the therapeutic target after 3 months (maximal reduction in LDL-C in one woman, - 83%). In addition, after 90 days Lp(a) levels were reduced in 4 patients (mean - 45 %).
**Conclusion.** Inclisiran leads to extensive LDL-C and Lp(a) reductions with high interindividual differences.

**Reference**
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## LDL-C TARGET ACHIEVEMENT AFTER ADDING BEMPEDOIC ACID IN A WOMAN WITH BIALLELIC LDL-RECEPTOR VARIANTS

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**Introduction.** Familial Homozygous Hypercholesterolemia (HoFH) an autosomal co-dominant disorder characterized by extreme elevations of LDL-C, tendon xanthomas and premature atherosclerotic cardiovascular disease (ACVD). It is consequent to mutations either in the LDLR, APOB or PCSK9 genes. Generally FH subjects partially respond to maximal statin therapy and LDL-C target achievement remains a challenge.
**Case report.** A 64-yo woman was diagnosed as HoFH 7 years ago after molecular confirmation. She carries biallelic mutants in LDLR (Arg300Gly and Arg303Trp). Untreated baseline lipids already at the age of 12 were TC 544, TG 100, HDL-C 52, LDL-C 472 mg/dL. During adolescence she was treated with cholestyramine at different doses, taken discontinuously over the years. She presented to our Lipid Clinic at age 30 with tendon xanthomas, hyperLpa (70 mg/dL), hypertension, smoking, family history of hyperlipidemia and CVD. Different drug treatments (statins,probucol, ezetimibe) had been tried with poor results, with LDL-C still at 273 mg/dl. More recently she received evolocumab 140 mg every 2-weeks added to rosuvastatin 40 mg+ezetimibe 10mg , followed by an LDL-C reduction to 121 mg/dl. At age 59 she had an acute antero-lateral MI and evolocumab was raised to 420 mg every 2 weeks, LDL-C reaching 69 mg/dl. In May 2023 bempedoic acid 180 mg was added and an LDL-C goal of 45 mg/dl was achieved after 2 months of treatment.
**Conclusion.** The addition of bempedoic acid is ideally suited to provide additional benefit in view of the excellent tolerability and rare occurrence of drug interactions. These data show the clinical efficacy of a therapeutic combination of bempedoic acid on top of PCSK9-inhibitor and a first- and second-level therapy with statin and ezetimibe (1), even in a complex FH patient with difficult target achievement.

**Reference**
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## REAL-EFFICACY OF BEMPEDOIC ACID IN REAL-WORLD DIFFERENT SETTING PATIENTS: BETTER THAN EXPECTED? EARLY PROMISING RESULTS OF A NEW SHARP ARROW TO OUR THERAPEUTIC BOW.

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**Background.** Several clinical studies, in particular the CLEAR Phase-3 "Galaxy" Studies (HARMONY, WISDOM, SERENITY, TRANQUILLITY and CLEAR FDC), have shown the cholesterol-lowering efficacy of Bempedoic Acid (BA) in different groups of patients, including statin-intolerant, with an average LDL-C reduction of about 24%.
**Aim.** To assess the efficacy of BA in monotherapy or in association with ezetimibe (FDC) in real-life patients, who needed an adjustment therapy both to achieve LDL target and/or due to intolerance to previous therapies. Therefore, we also compared our results with those reported in different setting patients as in the CLEAR Phase-3 studies.
**Methods.** 23 patients, (16F; 7M) have been included between April and August 2023 at our Center; 19 have had true, absolute/relative statin intolerance. Thirteen patients were treated with BA/ezetimibe, 3 with BA/ezetimibe association plus a low dose statin, 6 only with BA while the last one was in therapy with BA and PCSK9i. The primary endpoint was LDL-C reduction after the first month of therapy. Safety and tolerability have been assessed as self-patients reported. A subsequent analysis was carried out dividing the patients into their hypothetical CLEAR Phase-3 trial settings (13 CLEAR-TRANQUILLITY, 6 CLEAR-SERENITY and 4 CLEAR-FDC) and comparing the LDL-C reduction we observed with the relative clinical trial results.
**Results.** After only one month of treatment, patients showed an LDL-C reduction of 35.78% (±15.74): M=30.85±12.44; F=37.93±16.89.14 patients (58.33%) reached the LDL target, 9 did not, starting from a greater (about 40%) distance from the target due to absolute/relative statin intolerance. To be note that, of these latest 9, 80% obtained a >20% reduction, and in only 2 patients myalgia was still present. In our little and monocentric sample of real-world patients, the CLEAR-TRANQUILLITY-like patients obtained a reduction of 34.7% vs 28.5% expected, the SERENITY-like group showed a reduction of 31.3% vs 23.6% and the FDC-like group a result of -48% vs -38%, demonstrating a greater compliance and safety in real-life patients than traditional therapy.
**Discussion.** Despite the small sample, BA in real life has been shown to reduce LDL-C value alongside with a "clear" improvement in symptomatology and therefore compliance to the treatment. The results obtained in our "CLEAR-like settings" individually showed a reduction in LDL-C higher than that reached in the trials. The early data collected so far in our clinic (in some cases also at four months of treatment) confirmed the key role that BA is acquiring among hypercholesterolemic "difficult to treat" patients.

## IMPACT OF CONGENITAL UNCONJUGATED HYPERBILIRUBINEMIA ON LIPID METABOLISM AND ATHEROSCLEROSIS

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**Background.** Plasma Unconjugated bilirubin (UCB), a degradation product of heme catabolism that derives from the reduction of biliverdin (BVR), has antioxidant properties and is involved in diabetes, metabolic syndrome and cardiovascular disease protection. Although several studies indicated that the protective role of UCB/BVR is associated with anti-oxidant/anti-inflammatory effects, the molecular mechanisms are still poorly understood. Therefore, we investigate the effect of congenital hyperbilirubinemia during atherosclerosis on ApoE-/- background in standard chow diet.
**Materials and Methods.** Male ApoE-/-, ApoE-/-/Bvra-/- (knockout for the enzyme, biliverdin reductase, that reduce biliverdin to bilirubin), ApoE-/-/Ugt1-/- (knockout for the enzyme, glucuronosyltransferase, that convert unconjugated bilirubin to its conjugated form ready for degradation) and ApoE-/-/Bvra-/-/Ugt1-/- mice were fed on chow diet for 9 months. Blood, liver, spleen, bone marrow and mediastinal lymph nodes were collected and profiled by FACS analysis for immune cell subsets distribution. Atherosclerosis was profiled at the aortic level paralleled by the analysis of liver features.
**Results.** Our preliminary data show that ApoE-/-/Bvra-/- and ApoE-/-/ Bvra-/- /Ugt1-/- presented increased liver steatosis compared to ApoE-/- and ApoE-/-/Ugt1-/-, combined with increase macrophages and reduced NK regulatory cells infiltration. Cytofluorimetric analysis of the blood reveal a slight increase in neutrophils and a significant increase of cytotoxic natural killer cells in ApoE-/-/Bvra-/- and ApoE-/-/ Bvra-/- /Ugt1-/- mice compared to the other two groups. Finally, the quantification of aortic plaque formation indicated a similar aortic plaque area among the four experimental groups with comparable plasmatic cholesterol levels.
**Conclusions.** Overall congenital unconjugated hyperbilirubinemia in an experimental atherosclerotic setting doesn't affect plasma cholesterol and plaque formation. On the other hand, bilirubin shortage, or biliverdin accumulation, results in liver steatosis and in a worse immune cells profile in the liver. Further molecular analysis are ongoing to delineate the role of UCB on atherosclerosis development in high cholesterol diet.

## THE REDUCTION OF MONACOLIN K DOSAGE FROM 10 TO 2.9 MG RESULTS IN A LOSS OF EFFECTIVENESS OF THE LIPID-LOWERING THERAPY

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**Background.** Nutraceuticals can be a valid therapeutic alternative in certain classes of patients (B. Napolitano, SISA 2021, 2022). Since June 2022, European Union has mandated a reduction in the dosage of monacolin (<3 mg/day). There is currently no literature data regarding maintenance of therapeutic effectiveness on LDL levels with this dosage.
**Patients and Methods**. We evaluated efficacy [percentage reduction in LDL and percentage of achieving the therapeutic goal (<100 mg/dl) of a nutraceutical containing 2.9 mg of monacolin K (MK) in patients with moderate cardiovascular risk (SCORE >0.9 <5% at 10 years)], who were unwilling to take statins, compared to treatment with nutraceutical containing 10 mg. We evaluated 55 patients (35 females) consecutively attending outpatient clinic for dyslipidemia. All patients had been under treatment, for at least nine months (average 14.4±4.8 months), with a nutraceutical containing 10 mg of MK (Colenorm Plus®) and, since June 2022, they started taking nutraceutical containing 2.9 mg of MK. Before any lipid-lowering treatment, the values were: mean age 62.2±8.5 years, BMI 25.5±0.7, TC 202.6±29.3, HDL 53.2±10.7, LDL 125.4±31.7, TG 123±39.2.
**Results.** At baseline: BMI 26.7±3.7, TC 167.5±37.2, HDL 50.9±13.4, LDL 92.4±37.5 (-26% compared to no therapy), TG 120.3±59.7. The 63.64% of patients reached target <100 mg/dl. Six months after starting new therapy: BMI 26.9±3.5, TC 185.5±43.4, HDL 52.4±10.1, LDL 111.5±33.8, TG 126.5±31.8. LDL values decreased by 11.2% compared to no therapy but increased by 20.7% compared to 10 mg. Patients reaching the goal decreased to 27.27%.
**Conclusions.** Reduction of MK to 2.9 mg results in a significant lost in the efficacy on LDL levels and the achievement of the therapeutic goal compared to 10 mg. A new strategy for lipid-lowering nutraceuticals, based on the combination of 2.9 mg of MK with other lipid-lowering molecules, is necessary to achieve a good therapeutic response.

## DILI (DRUG-INDUCED LIVER INJURY), CARDIOVASCULAR RISK AND NEW PHARMACOLOGICAL WEAPONS. CASE REPORT OF FAMILIAL HYPERCHOLESTEROMY WITH STATIN INTOLERANCE

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60-year-old man, on therapy with Atorvastatin 20 mg 1cp / day for hypercholesterolemia from the age of 45. He was admitted to Internal Medicine for general malaise and acute hepatitis (ast 2000 U/K, alt 1500 U / L). Statin intolerance led to the use of ezetimibe combined with a previously prescribed balanced diet plan. Patients with clinical suspicion of familial hypercholesteromy (DLCN SCORE: 7). From 2015 to 2022, the patient underwent check-ups in the clinic every six months, where blood tests, clinical examination and instrumental evaluation for organ damage were viewed. The patient was affected by FAMILY HETEROZYGOTE HYPERCHOLESTROLEMIA with mutation of the LDLR and APOB gene, cardiovascular risk calculation: HIGH, this necessitates therapeutic continuity, periodic checks, cardio-metabolic follow-up. During the follow-up we witnessed a significant reduction in c-ldl values, reaching the therapeutic target according to ESC / EAS 2016 guidelines, (C-LDL <100 mg / dl, after prescription of Praulet (Alirocumab) 75 mg 1 fl sc every 14 days, subject to AIFA eligibility. With the introduction of the new evidence of cardiovascular risk present in the ESC / EAS 2019 guidelines, the patient appears to be out of the expected goal of about 30%, recommended LDL-c for risk class <70 mg/dl. In conclusion, the clinical case showed a therapeutic continuity aimed at minimizing the cardiovascular risk in a patient with FH. It places a magnifying glass on that minority of patients who report a severe adverse event while using statins and the possible use of other pharmacological approaches. In the panorama of innovative therapies against hypercholesterolemic dyslipidemia Bempedoic acid could be effective therapeutic alternative in patients not in range for c-ldl level, intolerant to statins. Specifically, a significant reduction in LDL cholesterol occurred after administration of Nustendi 180/10 mg (Bempedoic acid/ezetimibe) o.d, getting the therapeutic goal (LDL<70 mg/dl).

## WHOLE EXOME SEQUENCING IN VACCINE-INDUCED THROMBOTIC THROMBOCYTOPENIA (VITT)

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**Background.** In February 2021, few cases of unusual, severe thrombotic events associated with thrombocytopenia reported after vaccination with ChAdOx1 nCoV-19 (Vaxzevrya) or with Johnson&Johnson's Janssen vaccine raises concern about safety. The vaccine-induced thrombotic thrombocytopenia (VITT) has been related to the presence of platelet-activating antibodies directed against platelet factor 4.
**Objectives.** We investigated VITT subjects genetic background by a high-throughput whole exome sequencing approach in order to investigate VITT genetic predisposition.
**Methods.** Six patients (females of Caucasian origin with a mean age of 64 years) referred to the Atherothrombotic Diseases Center (Department of Experimental and Clinical Medicine, Azienda Ospedaliero-Universitaria Careggi, Florence) with a diagnosis of definite VITT underwent Whole Exome Sequencing (WES) analysis. WES analysis was performed on Illumina NextSeq500 platform.
**Results.** WES analysis revealed a total of 140,563 genetic variants. Due to VITT rare occurrence, we focused attention on rare variants. The global analysis of all high-quality rare variants did not reveal a significant enrichment of mutated genes in biological/functional pathways common to patients analysed. Afterwards, we focused on rare variants in genes associated with blood coagulation and fibrinolysis, platelet activation and aggregation, integrin-mediated signalling pathway as well as autoimmune thrombocytopenia. According to ACMG criteria, 31/112 (27.7%) rare variants were classified as uncertain significance variants (VUS), whereas remaining were likely benign/benign.
**Conclusion.** WES analysis identifies rare variants possibly favouring the prothrombotic state triggered by the exposure to vaccine. Functional studies and/or extension to a larger number of patients might allow a more comprehensive definition of these molecular pathways.

## CHARACTERIZATION OF LIPID AND LIPOPROTEIN PROFILE IN ALAGILLE SYNDROME

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**Background and Aim.** Alagille syndrome (ALGS) is a rare disease (1/70.000) variably characterized by chronic cholestasis due to paucity of intrahepatic bile ducts, peripheral pulmonary artery stenosis, vertebrae segmentation anomalies, characteristic facies, pigmentary retinopathy, and dysplastic kidneys. Most patients experience intractable pruritis and the presence of xanthomas, localized cholesterol and fats deposits under the skin caused by this liver dysfunction. Hypercholesterolemia is a well-known cardiovascular risk factor associated with atherosclerosis and arterial stiffening in liver disease. However, the role of lipid in ALGS-associated cardiovascular risk is unclear and lipids and lipoproteins are poorly characterized. Aim of this work was to characterize lipid and lipoprotein alterations in ALGS to identify potential pharmacological targets to prevent the onset and progression of cardiovascular and renal complications in these patients.
**Methods.** Six pediatric patients with ALGS were enrolled and lipid and lipoprotein profile was characterized.
**Results.** Three patients show high LDL-C, the presence of LpX and reduced HDL-C associated to alteration in subclasses distribution. Moreover, an impaired activity of LCAT enzyme is detected and can explain the increased free to total cholesterol ratio in these patients. Interestingly, the in vitro incubation with a synthetic LCAT activator is able to ameliorate lipid profile. However, these alterations are not observed in all patients; indeed, despite the broad clinical phenotype of ALGS and large number of mutations identified to date, there have been no genotype-phenotype correlations identified. The pattern and the degree of organ involvement may be different among patients, including those sharing the same mutation.
**Conclusions.** In our study, patients showing lipid abnormalities have more severe symptoms compared to patients with normal lipid profile. The correlation between lipids and the disease severity needs further studies to identify possible targets for therapeutic approaches aimed at preventing the onset and progression of cardiovascular and renal complications in ALGS.

## USE OF BEMPEDOIC ACID TO TREAT HYPERCHOLESTEROLEMIA IN TWO BROTHERS AFFECTED BY CHARCHOT-MARIE-TOOTH

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**Background.** Statins have been used for decades in the primary and secondary prevention of cardiovascular diseases. These drugs are known to be relatively safe yet associated with side effects, of which the most reported are related to the muscle such as myalgia, myositis or rhabdomyolysis. Particular caution is necessary in inherited peripheral neuropathy such as Charcot-Marie-Tooth disease. Statins have been classified in the uncertain/minor risk group in Charcot-Marie-Tooth patients. For these patients is now avaible theraphy with bempedoic acid. Bempedoic acid is an ATP citrate lyase inhibitor that targets cholesterol synthesis upstream of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme inhibited by statins but It is activated in the liver and not in most peripheral tissues, including skeletal muscle.
**Methods.** We reported two clinical cases of patients with Charcot-Marie-Tooth to assess efficacy and safety of Bempedoic acid. We evaluated lipid profile, liver and muscler enzyme at baseline and after 4 weeks of bempedoic acid treatment.
**Results.** We enrolled 2 patients of 25 and 39 years old respectively; both had nonalcoholic steatohepatitis, only one was hypertensive, no patient reported cardiovascular event or carotid atherosclerosis. Although both patients were on maximally tolerated hypolipidemic therapy with Ezetimibe, they have not achieved the desired LDL-C target. After 4 weeks treatment with Bempedoic acid, we observed a significant reduction in LDL-C levels (from 118 mg/dl to 58 mg/dl and from 115 mg/dl to 85 mg/dl, with an average reduction of about 40%). None reported any adverse event such us elevation of liver and muscular enzymes.
**Conclusion.** Hypercholesterolemia is common in patients with Charcot-Marie-Tooth disease because of decreased uptake of low-density lipoprotein (LDL). Although we currently have only two patients, Bempedoic acid has been shown to be aviable alternative therapy for both LDL-C level reduction and tolerance.

## IDENTIFICATION OF NEW SIDE CHAIN OXIDIZED STEROLS AS NOVEL LIVER X RECEPTOR AGONISTS WITH THERAPEUTIC POTENTIAL IN THE TREATMENT OF CARDIOVASCULAR AND NEURODEGENERATIVE DISEASES

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The nuclear liver X receptors (LXRα and β) may be potential therapeutic targets in cardiovascular and neurodegenerative diseases because of their key role in the regulation of lipid homeostasis and inflammatory processes. Among the mechanisms involved in the maintenance of macrophage cholesterol homeostasis, cholesterol efflux plays a crucial role. Specific oxy(phyto)sterols differentially modulate the transcriptional activity of LXRs, providing opportunities to develop new therapies. However, this development is precluded by unwanted side effects, such as hypertriglyceridemia and hepatic steatosis due to hepatic LXRα activation. The aim of this study was to investigate the effect of newly isolated oxyphytosterols from Sargassum Fusiforme and new synthesized side chain oxidized sterols on cholesterol efflux and on genes involved in this process.Cellular cholesterol efflux was evaluated with a radioisotopic cell-based assay on human hepatocellular carcinoma cell line (HepG2) after treatment with n=13 new LXRα/β agonists. Gene expression was assessed in HepG2 and in human astrocytoma cells (CCF-STTG1) by qPCR. We identified two sidechain 24-oxidized sterols (S2 and S6) with a high potency for LXRα/β activation. In detail, S2 increased cholesterol efflux from HepG2 by 54.3%, 15.2%, and 24.5% in presence of isolated APOA-I and HDL, and human serum (as cholesterol acceptor), respectively, while S6 increased cholesterol efflux by 9.3% and 6.7% in presence of APOA-I, and human serum, respectively. In addition, these sterols did not upregulate the expression of ABCA1 and ABCG1, but also they didn't promote the expression of SREBF1, SCD1, FASN or ACC1 in HepG2 cells, avoiding unwanted side effects, which are usual for synthetic pan-LXR agonist. In CCF-STTG1 cells, S2 and S6 slightly increased APOE, ABCA1, and ABCG1 mRNA levels. These results put the premises to identify and develop novels LXR-activating 24-oxidized sterols as potential therapeutic options in neurodegenerative and cardiovascular diseases.

## PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 (PCSK9) GENETIC DELETION ATTENUATES AMYLOID-β PATHOLOGY, NEUROINFLAMMATION AND IMPROVES COGNITIVE FUNCTIONS IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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**Background.** PCSK9, beyond the well-established plasma cholesterol regulatory activity, may be involved in Alzheimer's disease pathogenesis (AD), although the underlying mechanisms are not fully clarified. In this regard, we recently demonstrated in vitro that PCSK9 impairs brain HDL-mediated cholesterol transport from astrocytes to neurons and amplifies Amyloid β (Aβ)-induced neurotoxic effect. Hence, we aim to investigate the influence of PCSK9 on cognitive performances, Aβ burden, neuroinflammation, and brain lipids in a mouse model of severe AD.
**Methods.** We crossed 5XFADhet mice with PCSK9 KO mice to generate a mouse model of AD in which PCSK9 was genetically ablated. In 10-month old (mo) old mice, glial reactivity (both IBA1+-microglia and GFAP+-astrocytes) and Aβ burden (Thioflavin-S+- and Aβ+-plaques) were assessed by immunohistochemical analyses, while cognitive performances (spatial learning and memory) through the Morris water maze test. Cerebral cholesterol and hydroxysterols (OHCs) were quantified by fluorometric and LC-MS/MS analyses.
**Results.** PCSK9 ablation in 10 mo 5XFADhet mice decreased microgliosis and astrocyte reactivity, an index of attenuated neuroinflammation ($p<0.001$ vs 5XFADhet-PCSK9+ mice) in cortico-hippocampal brain areas, accompanied by a decrease in Aβ burden and improved cognitive performances ($p<0.05$ vs 5XFADhet-PCSK9+ mice, all). Conversely, PCSK9 ablation had minimal impact on brain cholesterol levels, while cerebral 24-OHC and 27-OHC concentration was unaffected.
**Conclusions.** This in vivo evidence suggests a protective role of PCSK9 genic deletion in 5XFAD, a severe mouse model of AD, against cognitive decline, Aβ pathology and neuroinflammation, only partially explained by the restoration of cerebral cholesterol homeostasis. Altogether, these results favour the premise of identifying PCSK9 as a pharmacological target for the development of novel therapeutic strategies for AD, for which no treatment is yet available.

## PCSK9: POST-TRANSLATIONAL MODIFICATION

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**Introduction.** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key player in modulating the number of low-density lipoprotein receptors (LDLR) at the hepatocytes surface. Therapies aimed at reducing free PCSK9 circulating levels decrease plasma LDL concentration by approx. 50 to 60% and, as a consequence, the risk of cardiovascular events. Monoclonal antibodies bind to PCSK9 preventing the interaction to LDLR and promoting its recycling and accumulation on the hepatocyte surface. The clearance of LDL-C particle accelerates from plasma, thus reducing their levels, meanwhile the plasma levels of PCSK9 increased 10-fold of which, on average, 10% was associated with LDL ($n=30$; $p<0.005$). Furthermore, PCSK9 undergoes post-translational modifications, including acetylation. Here we asked whether this affects binding PCSK9 to lipoproteins.
**Methods.** The OPTIPREP ultracentrifugation technique was used to isolate lipoproteins from the plasma patients before and after mAbs therapy. The 23 fractions were collected and their cholesterol, triglycerides and PCSK9 contents were analyzed by colorimetric and ELISA assays. The presence of post-translational changes in PCSK9 were studied by immunoblotting using the antibody anti acetyl-lysine (1:600).
**Results.** The results are reported in a graph in which 2 peaks (PCSK9-bound to LDL and PCSK9 free) on PCSK9 curve and 1 major peak (LDL free) on cholesterol curve are observed. The fractions contained in every single peak were pooled and analyzed by immunoblotting. An increment in PCSK9 acetylation was observed after mAbs therapy in the PCSK9-bound LDL pool.
**Conclusion.** Acetylated PCSK9 appears to preferentially associate to LDL in circulation. Further studies are needed to investigate the biological activity of acetylated PCSK9.

## LONG TERM FOLLOW-UP AND TREATMENT OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA IN MODENA LIPIGEN COHORT

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**Introduction.** Familial hypercholesterolemia is a genetic disorder characterized by high levels of low-density lipoprotein cholesterol (LDL-C) and an increased risk of atherosclerotic cardiovascular events. Strategies to reduce LDL-C are essential to improve the prognosis of patients affected by familial hypercholesterolemia. The advent of new lipid-lowering drugs has improved the management of these patients in recent years. However, real-life studies are needed to confirm the impact of these pharmacological advancements in clinical practice. This retrospective observational study aims to evaluate the trend in the use of lipid-lowering medications and the achievement of LDL-C therapeutic targets in a cohort of patients with familial hypercholesterolemia followed at a tertiary level LIPIGEN Center.
**Materials and Methods.** A total of 413 patients diagnosed with familial hypercholesterolemia based on clinical and/or genetic criteria were enrolled in this retrospective observational cohort study (mean age 49 [33-59] years, 49% male, genetic diagnosis 73%, maximum LDL-C 250 [210-309] mg/dl, secondary cardiovascular prevention 15.7%). All patients were followed at the LIPIGEN Center in Modena. Clinical and laboratory data related to lipid profiles, cardiovascular events, and pharmacological treatment were collected at baseline (first visit to the Center) and during a median follow-up of 39 [19-55] months. LDL-C therapeutic targets were defined according to the 2019 ESC/EAS guidelines.
**Results.** At baseline, LDL-C levels were 162 [127-208] mg/dl. Only 60% of the patients were treated with statins, with 49.6% of them receiving high-intensity statin-based therapy. Ezetimibe and PCSK9 inhibitors were used in 34.9% and 1.7% of cases, respectively. Only 4 patients (1.0%) had a guideline-recommended LDL-C level. During follow-up, there was a significant increase in the prescription and association of lipid-lowering medications: statins (89.1%), with 58.2% receiving high-intensity therapy, ezetimibe (75.5%), and PCSK9 inhibitors (31%) (all $p<0.001$). This led to a 48% reduction [25.9-63.6]% in LDL-C levels compared to baseline, reaching a median of 88 [59-109] mg/dl ($p<0.001$) and allowing the achievement of the target in 124 patients (30%) ($p<0.001$). The use of the triple combination of statin, ezetimibe, and i-PCSK9 in 113 patients (27.4%) resulted in the therapeutic target being reached in 88.5% of cases, with median LDL-C levels of 45 [39-57] mg/dl. Patients who achieved the therapeutic target during the follow-up were significantly older and more frequently male, probands, with a genetic diagnosis of Familial Hypercholesterolemia, a family and/or personal history of cardiovascular events, higher LDL-C burden, and a higher prevalence of cardiovascular risk factors (smoking, hypertension, diabetes, visceral adiposity). Fourteen patients (3.4%) experienced incident cardiovascular events during the follow-up, with age, LDL-C burden, personal history of previous cardiovascular events, hypertension, diabetes, and visceral adiposity emerging as predictors of incident cardiovascular events.
**Conclusions.** The treatment of patients with Familial Hypercholesterolemia has improved over time. However, a considerable percentage of individuals fail to achieve the LDL-C therapeutic target. The use of triple combination therapy with a statin, ezetimibe, and i-PCSK9 represents the best strategy for LDL-C reduction, allowing the target to be achieved in the majority of FH patients at higher cardiovascular risk.

## EPICARDIAL ADIPOSE TISSUE AND PERICARDIAL ADIPOSE TISSUE AS CELL MODELS TO ASSESS PATIENT RESPONSIVENESS TO THERAPEUTICS

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**Background.** The abundance of epicardial and pericardial adipose tissue is associated with several cardiac risk factors and higher carotid intima-media thickness, suggesting a pathogenic role of epicardial adipose tissue and pericardial adipose tissue in the development of atherosclerosis. Under obesity conditions, a shift in metabolic functions toward inflammatory and pro-atherogenic phenotypes occurs in both fat depots. Curbing epicardial and pericardial adipose tissue dysmetabolism and associated inflammation may represent a novel therapeutic target to prevent coronary atherosclerosis.
**Purpose.** Development of a feasible and efficient method for isolation of adipocytes from human pericardial adipose tissue and evaluation of adipocyte response to therapeutics and micronutrients.
**Methods.** Pericardial adipose tissue was collected from coronary patients undergoing surgery for coronary stenosis and from patients undergoing aortic or mitral valve surgery and immediately enzymatically processed. Isolated adipocytes were morphologically and molecularly characterized. Cell responsivity was evaluated by exposure to inflammatory stimuli or to docosahexaenoic acid, a well-known cardio-protective fatty acid.
**Results.** We obtained pure cultures of adipocytes that can be sub-cultured for several days without losing viability and retaining the ability to respond to stimuli. Basal expression of inflammatory genes in adipose cells was higher in coronary patients than in aortic and mitral surgery patients. Exposure of adipocytes to Tumor necrosis factor alpha significantly induced the messenger and protein expression of monocyte chemoattractant protein-1 and Interleukin 6 ($p<0.05$), while downregulated the expression of uncoupling protein-2 and Peroxisome proliferator-activated receptor gamma ($p<0.05$). On the other hand, the exposure of adipocytes to DHA resulted in a downregulation of monocyte chemoattractant protein-1, CXCL10 and Interleukin 6 expression ($p<0.05$) and in the upregulation of Uncoupling protein-1 and -2 and Peroxisome proliferator-activated receptor gamma ($p<0.05$).
**Conclusion(s).** Our data propose a new efficient method for isolating adipocytes from PAT and for using them as a bio-reactor to test differences in different cardiovascular conditions.

## RESISTANCE TO DRUG THERAPY MADE IT POSSIBLE TO IDENTIFY A DOUBLE MUTATION OF LDLR GENE IN HETEROZYGOSITY

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We report the case of a 54-year-old woman, smoker since she was 25, uncontrolled diet, no physical activity and an acute myocardial infarction at the age of 43. She had a positive family history for early cardiovascular events with a familial hypercholesterolemia diagnosis since she was 33 (TC 603 mg/dL, LDL – C 440 mg/dL; hypercholesterolemic mother, sisters, brothers, nieces and children). The children were heterozygous for the mutation c.1211C>T(p.Thr404Ile) in exon 9 of the LDLR gene and the same mutation was found in the proband by Sanger investigatio. She tried statins with no benefit. In 2017 evolocumab was associated. In 2021 therapy with ezetimibe, rosuvastatin, fenofibrate and levolocumab was not sufficient to control values (LDL-C 324 mg/dL). In 2022 she started lomitapide and a NGS screening was performed in consideration to the different pharmacological effect and clinical trend compared to other family members. A digenic heterozygous mutation c.241C>T in exon 3 of the LDLR gene were found in addition to the previous one. She is in good clinical conditions, good laboratory response (TC 215 mg/dL, LDL-C 114 mg/dL) with lomitapide, evolocumab and statin.

**Methods.** Library preparation and sequencing by synthesis on the Illumina platform (kit TruSight One Expanded o TruSight Cardio and sequencing on NEXTSEQ550). The bioinformatic analysis involves the use of BWA Aligner or DRAGEN Enrichment software. The sequences are aligned to the human reference genome GRCh37 which allows the calling of single nucleotide and structural variants. Illumina Variant Interpreter software or TGEX Software was used for variant analysis. For the selection of genes associated with the clinical indication, the OMIM, PanelApp and Orphanet databases were used. Only variants with read depth >20x and adequate quality parameters were considered Variants are annotated according to the HGVS nomenclature and classified according to the American College of Medical Genetics and Genomics guidelines. Analysis of the impact of the variants on the encoded protein was performed with the following software: SIFT, PolyPhen2, GERP, Phylo P, PhastCons, Mutation Taster, Mutation Assessor, DANN, AlignGVGD and Grantham Score. For variants that impact the splicing mechanism, the Alamut software is used.

The genetic analysis allowed us to find:

- The variant c.1211C>T in exon 9 of LDLR gene, with consequent replacement of the threonine with isoleucine in position 404. This variant, present in heterozygosity in the patient, is classified as pathogenic from the analysis software and from Varsome, with conflicting interpretation from ClinVar. The variant is reported in dbSNP, while it is absent in GnomAD. The variant affects a highly conserved nucleotide, falls in the "LDL receptor class B1" functional domain of the protein and is reported in the medical literature in association with familial hypercholesterolemia (1);
- The variant c.241C>T in exon 3 of LDLR gene, with consequent replacement arginine with cysteine in position 81. This variant, present in heterozygosity in the patient is classified as uncertain meaning from the analysis software, such as pathogenetic by Varsome and with conflicting interpretation by ClinVar. The variant is reported in dbSNP and in GnomAD. The variant affects an averagely conserved nucleotide, falls in the "LDL receptor class A2" functional domain of the protein and is reported in the medical literature as probably pathogenetic in association with familial hypercholesterolemia (2).

**Conclusions.** Resistance to drug therapy made it possible to identify a double pathogenic mutation affecting LDLR gene, both in heterozygosity leading to a LDLR digenic condition. This finding is compatible with the patient's clinical presentation.

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## EFFICACY AND SAFETY OF MONOCLONAL ANTIBODIES AGAINST PCSK9: REAL-LIFE DATA

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**Background.** Hypercholesterolemia is one of the most important cardiovascular risk factors. Among the new lipid-lowering drugs we include monoclonal antibodies against PCSK9, as Evolocumab and Alirocumab, that have been showed in several trials as an effective therapy against hypercholesterolemia. Anyway, real life studies are important to monitor efficacy and safety on patients.

**Purpose of the Study.** Our study was aimed to evaluate the response to lipid-lowering therapy (LLT) with monoclonal antibodies in patients belonging to different cardiovascular risk classes and to relate it to background therapy.

**Materials and Methods.** In this monocentric retrospective study, we included 164 patients currently followed by the Dyslipidemia Clinic of "D'Annunzio" University and who have started LLT with PCSK9i monoclonal antibodies within the last 5 years: 105 (64%) Alirocumab and 59 (36%) Evolocumab. Considering basic therapy, 66% of patients receiving Alirocumab, vs 68% of those receiving Evolocumab (globally 66% of all patients included in our study) had already maximal treatment (statin + ezetimibe) when started PCSK9i.

**Results.** Considering all patients included, 60% reached LDL-C target based on their own cardiovascular risk class. Better results were observed among patients treated with Alirocumab (62%) than with Evolocumab (56%). It must be considered that: the two groups have different numbers; Titration and optimization of therapy (mainly due to casual true or partial statin intolerance) has been greater in patients treated with Alirocumab (72%) compared to Evolocumab (55%). Anyway, the most important result we have observed is that no cardiovascular events occurred during observation period since patients started PCSK9-i.

**Conclusions.** Clinical efficacy and safety of LLT with monoclonal antibodies is confirmed by our real-life data. Our data also highlight the need of a background optimized and combination therapy to ensure LDL-C target achievement.

## PLATELET TO GAMMA-GLUTAMYL TRANSFERASE RATIO: A NOVEL EASY-TO-USE TOOL TO PREDICT HIGH LIPOPROTEIN(A) LEVELS

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Lipoprotein(a) – Lp(a) – is a lipid molecule with inflammatory, atherogenic and pro-thrombotic effects. The management of Lp(a) levels represents an emerging challenge for lipidologist: in fact, the evaluation of Lp(a) levels covers a clinical significance in the assessment of residual cardiovascular risk, due to its well-established correlations with atherosclerotic cardiovascular disease (CVD), for which Lp(a) can be considered an independent and causal risk factor. The most recent American and European guidelines suggest a cut-off value of Lp(a) concentration ≥50 mg/dL to identify subjects with high cardiovascular risk.
The aim of this retrospective study was to find a predictor of high Lp(a) levels, in order to easily screen patients for high cardiovascular risk. We retrospectively enrolled subjects visited in our Internal Medicine Unit during Day-Service access, for which Lp(a) dosage was available. Clinical and biochemical data were collected: taking into account that Lp(a) plays a role in inflammation and that its synthesis depends on hepatic functionality, we collected data about blood count, liver function tests, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).
Results were expressed as median and interquartile range (IQR). Kolmogorov-Smirnov test was used to assess sample normality; Spearman's Rho test was used to find possible relationships between Lp(a) and other laboratory findings. Receiver operating characteristic (ROC) curve was used to identify a valid predictor of high Lp(a) values. A p-value less than 0.05 was considered statistically significant. Statistical analysis was made with SPSS Software (version 26). 245 patients (113 females, 46,1%) were enrolled. Median age was 65 (IQR 19); the sample was heterogeneous about comorbidities: about 50% suffered from diabetes, 30,2% from chronic renal failure, 60,4% from peripheral artery disease; 67,8% had arterial hypertension, 70,6% dyslipidaemia; less than 10% suffered from ischemic heart disease, venous thromboembolism, autoimmune diseases, cancer, ischemic stroke. Median Lp(a) level was 12,8 mg/dl (IQR 30,45). Results showed a positive statistically significant relationship between Lp(a) and platelet count (R=0,168, p=0,009) while no further correlations were found with blood count. Moreover, a positive statistically significant correlation between Lp(a) and ESR (R=0,144; p=0,024) was found, while the relationship with CRP wasn't significant (R=-0,005; p=0,943). Finally, significative inverse correlations of Lp(a) with aspartate aminotransferase (AST) (R=-0,155; p=0,015), alanine aminotransferase (ALT) (R=-0,143; p=0,025) and gamma glutamyl transferase (GGT) (R=-0,160; p=0,012) were found, while correlation with alkaline phosphatase wasn't statistically significant (R=0,043; p=0,507).
Moving from these findings, we decided to create a new predictive index of Lp(a) levels in the form of a mathematical fraction, choosing for the numerator a variable with a positive correlation with Lp(a) and for the denominator a variable with a negative one. In this way, platelet to AST ratio, platelet to ALT ratio and platelet to GGT ratio were tested with ROC curve analysis in order to find an ideal predictor of Lp(a) levels equal or greater than 50 mg/dl.
Results showed a non-statistically significant test for platelet to AST ratio (area under the curve -AUC- 57%; p=0,118; 95% confidence interval -IC - 48-66) and for platelet to ALT ratio (AUC 58%; p=0,072; 95% CI 49-67). In contrast, platelet to GGT ratio has revealed to be useful to predict Lp(a) levels equal or greater than 50 mg/dl with a best cut-off value of 3337 (AUC 60%; p=0,031; 95% CI 51-68; 98% sensitivity, 90% specificity).
Concluding, our research shows, for the first time, a new way to screen patients for high Lp(a) levels, with the help of a very easy-to-use tool. Given the need to confirm data and improve diagnostic accuracy of the test, platelet to GGT ratio could be used in the context of clinical lipidology for the assessment of residual cardiovascular risk.

## MANAGEMENT OF DYSLIPIDEMIAS IN TELEMEDICINE: AN EXPERIENCE AT A UNIVERSITY HOSPITAL

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**Background.** The pandemic has highlighted all the fragilities of our healthcare system, including the socioeconomic and geographical disparity in access to services, the reduced integration between hospital services and community services, the very high waiting times for access to some health services. Overcoming these fragilities has become a priority in our country. Among the actions tobe implemented to develop digital healthcare of the future, also thanks to Telemedicine, there is measuring the results obtained from the local Telemedicine projects carried out so far so as to guide the choices of policy-makers and various professionals, also through sharing of experiences. Ameta-analysis conducted in diabetic patients has shown that telemedicine allow a greater reductionin glycosylated hemoglobin, while this was not observed on blood pressure.
**Methods.** Below we report the experience of the Telemedicine Operational Structure activated at the University Hospital "Renato Dulbecco (ex-Mater Domini)" of Catanzaro, where the Clinical Nutrition teleclinic is active, which deals with the management of dyslipidemic patients with and without obesity.
**Results.** In the last year, we carried out n.79 telemedicine visits, with a mean number of follow-upvisits of 2. The dyslipidemic patients were n.21, and 56% were women. At the first tele health visit,LDL was 110±37 mg/dL, triglycerides were 106±57 mg/dL, CPK was 126±67 mg/dL. At the second visit of telemedicine, only n. 3 patients were lost, and the LDL value was 96±49 (~ -14)mg/dL, triglycerides were 100±43 mg/dL, and CPK was 117±37 (~ -9) mg/dL. Then we excluded FH patients, at the follow-up LDL was 81±21 mg/dL. In comparison with in-person out patient visits, no significant differences were highlighted on lipid serum parameters.
**Conclusions.** Telemedicine allows us to break down barriers, and is as effective as the face-to-faceapproach. Patients referred good adherence to therapy with clinical benefits.

## SEVERE DELAYED SYSTEMIC CUTANEOUS REACTIONS IN PATIENTS TREATED WITH EVOLOCUMAB OR ALIROCUMAB

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Evolocumab and alirocumab are monoclonal antibodies (mAbs) that inhibit the proprotein convertase subtilisin/kexin type 9 (PCSK-9), a mechanism that leads to very significant reductions of LDL-cholesterol levels in blood. In long-term phase-3 clinical trials, the adverse events reported with these compounds have been comparable to those observed with placebo, except for more frequent injection-site reactions in the active treatment branch. These drugs have shown very good safety and tolerability profiles also in real-world clinical practice (PMID: 30053327; PMID: 36506552). Systemic cutaneous hypersensitivity reactions have been reported but these side effects have generally been mild to moderate (PMID: 31292332; PMID: 34188988; PMID: 35818959). In this report we describe and show pictures of three cases of delayed severe widespread cutaneous adverse reactions, occurring 1 to 3 years after treatment initiation with anti-PCSK-9 mAbs. These side effects were presumably induced by the antibodies, compelled their discontinuation and resolved after drug interruption and symptomatic treatment. Notably, all three patients had a remote history of minor allergic reactions: cutaneous pruritus with proton pump inhibitors (patient 1), atopic dermatitis in skinfolds during childhood and adolescence (patient 2) and allergic rhinitis upon exposure to cat hair and almonds (patient 3). Factors that strongly suggest a causal relationship between the compounds and the adverse effects are:

1) absence of a history of similar cutaneous reactions before treatment with these compounds;
2) temporal association between treatment doses and emergence or worsening of symptoms,
3) lack of any change in concomitant therapies or exposure to any other known allergen or toxic substance;
4) more or less rapid relief of symptoms after treatment discontinuation.

Large pharmacovigilance data are needed to understand whether a personal history of minor hypersensitivity should be included in the individual cost-benefit appraisal when treatment with an anti-PCSK-9 mAb is being considered.

## ANGPTL3 SILENCING AND HEPATIC FAT ACCUMULATION: AN IN VITRO 2D AND 3D MODEL TO INVESTIGATE THIS EFFECT

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**Background and Aim.** ANGPTL3 is an hepatokine acting as negative regulator of lipoprotein lipase (LPL) and targeted by multiple therapies. Vupanorsen, ANGPTL3 directed antisense oligonucleotide, has been discontinued from phase 2b clinical trial due to an unexpected increase in liver fat fraction. The aim of this project is to shed new insights on the intracellular mechanism causing fat accumulation.
**Methods.** We utilized hepatocarcinoma Huh7 cells treated with siRNA-ANGPTL3, human recombinant ANGPTL3 (hrecANGPTL3) or the combination of the two (siRNA+hrec). By western blot, Oil red-O, biochemical assays and ELISA assays, we analysed the expression of genes and proteins involved in lipid metabolism.
**Results.** Oil red-O analysis demonstrated that lipid content increased after ANGPTL3 silencing (5.89±0.33 fold), hrecANGPTL3 administration (4.08±0.35 fold) and the combination of both (8.56±0.18 fold) compared to untreated cells. We observed an increase in pro-SREBP1 and fatty acid synthase, respectively by 100% and by 45% after siRNA-ANGPTL3 and combined treatment. Cellular LPL activity doubled with siRNA-ANGPTL3 treatment as expected. No differences in secreted ApoB and total cholesterol were found in the different conditions.
**Conclusions.** Efficient lipid accumulation following gene silencing emerged from our experiments, mirroring what was seen in patients treated with vupanorsen. The investigation led us to hypothesize an increase in triglyceride synthesis and lower secretion rate when ANGPTL3 is silenced. Moreover, silencing and administration appear to behave in an addictive manner, following two different pathways that are currently under analysis. These results suggest a possible hepatic role of ANGPTL3 which is still unknown and must be further studied.

## CHARACTERIZATION OF REPLICATIVE AND DOXORUBICIN-INDUCED SENESCENCE MODELS IN VASCULAR SMOOTH MUSCLE CELLS

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**Background.** Cellular senescence is characterized by progressive exhaustion proliferation, senescence-associated secretory phenotype (SASP), high level of oxidative stress and dysfunctional mitochondria. Accumulation of senescent vascular smooth muscle cells (VSMCs) contributes to aging as well as cardiovascular disease. Senescent VSMCs are present in atherosclerotic plaques and contribute to their instability.
**Aim.** Since there are no unanimously agreed senescence markers in human VSMCs, we aimed at establishing the molecular signatures of replicative senescence (RS) and doxorubicin-induced senescence in human VSMCs by means of multi-biomarkers approaches, performing an in-deep cellular morphological analysis and evaluating the expression of manually selected senescence- associated genes.
**Materials and Methods.** Human aortic VSMCs were serially passaged to represent different stages of RS and used from 5th to 7th passages (young cells) and from 15th to 17th passages (old cells), and to perform doxorubicin-induced senescence model we treated young cells with 100nM of doxorubicin for 48 hours. We measured SA-β-gal activity (a marker of senescence), genes and proteins expression by qPCR and western blot analysis, cell proliferation by cell counting, cell cycle, reactive oxygen species (ROS) production and mitochondrial membrane potential by flow cytometry, mitochondrial function by seahorse, mitochondrial morphology by confocal microscopy and morphological and nuclear changes by immunofluorescence.
**Results.** Both RS and doxorubicin-induced senescence cells showed more than 50% of SA-β-gal positive cells compared to 20% of young cells, a flattened appearance, and enlarged and irregular nuclei, with LMNB1 and HMGB1 expression downregulated, indicating an altered nuclear membrane. The expression of cell cycle inhibitors (p21/p16) was upregulated in both models, in particular we observed a G1-cycle arrest in RS instead a G2-cycle arrest in doxorubicin-induced senescence model. Then, RS significantly induced SASP molecules (e.g. IL1β, IL6, IL8, MMP-3) expression, instead in doxorubicin-induced senescence model we observed higher expression of inflammatory markers only after 3 days of recovery. Finally, the two senescence models showed mitochondrial dysfunction characterized by a decrease in respiratory capacity together with a decreased mitochondrial membrane potential, accompanied by increased production of mitochondrial ROS. Furthermore, both senescence models showed altered mitochondria morphology with a high percentage of fragmented mitochondria compared to the high proportion of elongated mitochondria in young VSMCs.
**Conclusions and Future Perspectives.** We performed a detailed characterization of RS and doxorubicin-induced senescence model in VSMCs underling their similarities and differences. Then, these models will be suitable to identify potential anti-aging factors (e.g. senolytics or senomorphics) as a therapeutic approach in aging and aging-related diseases, or analyze the effects of pro-atherosclerotic risk factors (i.e. cigarette and tobacco products smoke) on senescence.

## TRYING TO REACH THE LOW-DENSITY LIPOPROTEIN CHOLESTEROL TARGET: MONOCENTRIC RESULTS AFTER TWO YEARS OF REAL-WORD TREATMENT OF HIGH- AND VERY-HIGH-RISK PATIENTS ALREADY ENROLLED IN THE SANTORINI STUDY

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**Background.** Results from SANTORINI, an observational, prospective study that documented the use of lipid-lowering therapies (LLTs) in 9044 adults at high or very high cardiovascular (CV) risk between 2020 and 2021 in 14 European countries, showed that only 20.1% of patients achieved risk-based LDL-C goals recommended by 2019 ESC/EAS Guidelines.
**Aim and Methods.** The aim of this study was to evaluate the target distance among the 27 SANTORINI patients enrolled from Chieti (66.7% women, mean age 61.67±12.49 years, 77.8% at high CV risk) according to ESC/EAS Guidelines at 2-year follow-up and the efficacy of LLTs prescribed.
**Results.** Total cholesterol (TC) and LDL-C significantly decreased from baseline (TC:200.4±66.1, LDL-C:120.6±59.8 mg/dL) to 1-year (TC:165.5±62.1, LDL-C:86.8±46.6 mg/dL) and 2-year follow-up (TC:155.8±73.0, LDL-C:78.4±72.3 mg/dL; TC p=0.017, LDL-C p=0.014). Despite the absence of statistical significance, a reduction in HDL-C and triglyceride levels was found at 1 year and maintained at 2 years. At 2-year follow-up there was a reduction in high-dose statin use, and an increasing use of PCSK9-i (p=0.013) and low-dose statin. Combination therapy was used in 66.7% of patients at baseline, 77.8% at 1 year and 81.4% at 2 years, percentages higher than those reported in Santorini study (Italy 33%, overall 25.8%) .53.8% of patients achieved the LDL-C target at 2 years, while 14.8% were at target at baseline and 34.6% at 1 year (p=0.002) (mean in global SANTORINI group = 20.1%). For patients who did not reach LDL-C goals, the distance from target decreased progressively from 45 mg/dL (IQR 19.75-100.50) at baseline to 39 mg/dL (5.50-80.50) at 1 year and 21.5 mg/dL (2.75-55.75) at 2 years.
**Conclusion.** If SANTORINI study highlighted a critical discrepancy between Guidelines recommendations and clinical practice, our results are encouraging. Furthermore, a significant association between target achievement and exclusive management at the Reference Center has not been demonstrated (at least in our group), suggesting an increasing general practitioners' awareness of the problem in the last year.

## PCSK9 AS A PROGNOSTIC BIOMARKER OF CARDIOVASCULAR EVENTS IN PATIENTS WITH TYPE 2 DIABETES: A 16.8-YEAR FOLLOW-UP STUDY.

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Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality, being twofold to fourfold more common in patients with type 2 diabetes mellitus (T2DM) than in individuals without diabetes. However, despite this decade-old knowledge, particularly challenging remains the identification of a specific prognostic risk biomarker. The present study was aimed at testing the hypothesis that fasting serum proprotein convertase subtilisin/kexin type 9 (PCSK9) levels could be prognostic for major cardiovascular events (MACE) and all-cause mortality, in patients with a diagnosis of T2DM followed for a median of 16.8 years (n= 529 Caucasians). Serum PCSK9 median levels were 259.8 ng/mL, being higher in women (median: 271.3 ng/mL) compared to men (median: 246.9 ng/mL) and increasing even more in the presence of a complication (e.g., diabetic kidney disease (median: 285 ng/mL)). PCSK9 positively correlated with markers of blood glucose homeostasis (e.g., HbA1c, fasting insulin and HOMA-IR) and the atherogenic lipid profile (e.g., non-HDL-C, apoB and remnant cholesterol). Serum PCSK9 predicted new-onset of MACE (myocardial infarction, cardiac arrest, cardiogenic shock, life-threatening arrhythmia, or stroke), either fatal or non-fatal, only in women (Odd Ratio: 2.26, 95% confidence interval 1.12 - 4.58) and all-cause mortality only in men (Hazard Ratio: 1.79, 95% confidence interval 1.13 - 2.82).In conclusion, considering that up to two-thirds of individuals with T2DM develop ASCVD in their lifetime, the assessment of circulating PCSK9 levels can be envisioned within the context of a biomarker-based strategy of risk stratification. However, the sex difference requires an urgent need to develop sex-specific risk assessment strategies.

## STATIN INTOLERANCE AND QUALI-QUANTITATIVE CHARACTERISTICS OF MUSCLE MASS

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**Introduction.** Physical activity reduces the risk of cardiovascular disease. However, statin-associated musculoskeletal side effects can be exacerbated by physical activity. As consequence, a reduced statin use occurs in individuals who would benefit from statin therapy. This study explores interactions between statin/exercise and muscle damage in vitro and offers, for the first time, a new view in the physiopathological mechanisms of the statin intolerance.
**Methods.** For the in vitro study, we evaluated the effect of Atorvastatin and Rosuvastatin on muscle cells (C2C12), using caffeine to provide an exercise-like model. We performed all tests on differentiated myotubes. In particular, we evaluate the proliferation, myotubes diameter and gene expression of atrophy markers (Murf-1 and Atrogin-1) and isoforms of Myosin (MyHC-I, MyHC-IIa, MyHC-IIb and MyHC-IIx).
**Results.** In in vitro, we showed a high proliferative effect of Rosuvastatin on cells than caffeine (10 nM and 20 nM Rosuvastatin vs Caffeine, $p<0.0001$ and $p=0.03$, respectively). The combination of Rosuvastatin and Caffeine prevents reduction in myotube size (10 nM and 20 nM Rosuva vs Caffeine, $p=0.001$ and $p<0.001$, respectively) and markers of muscle atrophy (Murf-1: 20 nM Rosuva vs Caffeine, $p=0.02$), as well as modulate myosin isoforms in a caffeine-induced exercise model.
**Conclusions.** A proliferative and preventive effect of Rosuvastatin on muscle damage is conceivable, as showed by the increased expression of all myosin isoforms in comparison with exercise (caffeine) alone and control. Further studies are needed to better understand the mechanism by which statins increase muscle proteins expression.

## HIGHER CIRCULATING LEVELS OF NON-ESTERIFIED FATTY ACIDS ARE ASSOCIATED WITH FASTER KIDNEY FUNCTION DECLINE IN POST-MENOPAUSAL WOMEN WITH TYPE 2 DIABETES: A PILOT PROSPECTIVE STUDY

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**Aims.** There is currently little and inconsistent evidence regarding the possible adverse effects of circulating levels of non-esterified fatty acids (NEFA) on kidney function decline in patients with type 2 diabetes mellitus (T2DM).
**Materials and Methods.** We followed for a median of 4.6 years 85 post-menopausal women with T2DM and preserved kidney function at baseline. Serum NEFA concentrations were measured using an enzymatic colorimetric method. Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.
**Results.** Enrolled patients had a baseline mean eGFR CKD-EPI of 83±12 mL/min/1.73 $m^2$, and a median serum NEFA concentration of 662 uEq/L (interquartile range: 524-842 uEq/L). During the follow-up period, 13 patients developed kidney function decline at follow-up (defined as an eGFR CKD-EPI decline ≥30% from baseline). In Cox proportional hazards regression analysis, higher circulating levels of NEFA were significantly associated with increased risk of developing kidney function decline (adjusted-hazard ratio 3.67, 95% CI 1.64-8.22, p<0.001; for each 1-SD increment, i.e., 262 uEq/L), even after adjustment for waist circumference, hemoglobin A1c, C-reactive protein, HOMA-estimated insulin resistance, hypertension, dyslipidemia, microalbuminuria, baseline eGFR CKD-EPI, as well as temporal changes in HbA1c levels or in the use of renin-angiotensin system inhibitors over the follow-up.
**Conclusion.** The findings of our exploratory prospective study show that in post-menopausal women with T2DM and preserved kidney function at baseline, higher circulating levels of NEFA were strongly associated with a faster kidney function decline, even after adjustment for established renal risk factors and potential confounders.

## LIPID LOWERING AFTER A SINGLE INCLISIRAN ADMINISTRATION: PRELIMINARY DATA FROM A REAL-WORLD EXPERIENCE

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**Background.** Inclisiran is a small interfering RNA (siRNA) that prevents PCSK9 synthesis, thus lowering low-density lipoprotein cholesterol (LDL-C). In randomized trials, Inclisiran demonstrated a reduction in LDL-C levels by approximately 50%. The aim of this observational study was to assess the efficacy and safety profile of Inclisiran for management of high or very-high cardiovascular risk patients in a real-world setting.
**Methods.** We performed a retrospective analysis of the first 32 patients who received a single dose of Inclisiran at two Lipid Units (Verona and Legnago) between September 2022 and May 2023. Baseline lipid profile was taken prior to start of treatment and repeated at 1 month and 3 months follow-up.
**Results.** Of the 32 patients (23 males, 9 females, mean±DS age 63.4±9.8 years), 25% had familial hypercholesterolemia (FH), 75% coronary heart disease, 19% stroke, 50% peripheral vascular disease, 78% hypertension, 41% impaired fasting glucose, 28% diabetes mellitus (DM), 19% chronic kidney disease (CKD), 38% multiple cardiovascular events, 28% statin intolerance. Baseline mean±DS LDL-C levels were 122.6±58.1 mg/dl. 41% of patients had hyperlipoprotein(a), median baseline levels 80 mg/dl [IQR 64-106]. Following a single subcutaneous Inclisiran injection, mean LDL-C levels fell by 60.7% at 1 month and 54.6% at 3 months. LDL-C target in very high-risk patients (<55 mg/dl) was reached in 70% at 3 months. A greater reduction in LDL-C levels was experienced in statin users (-59.3% vs -48.5%) and patients with DM (-72.7% vs -48.2%). In FH patients a lesser decrease was noticed (-52.7% vs -55.7%). LDL-C reductions were consistent among sex, presence of CKD and hyperLp(a). No side effects were reported.
**Conclusion.** Inclisiran substantially reduced LDL-C levels in patients with high or very-high cardiovascular risk in a real-world setting, with results comparable to those reported in clinical trials and good tolerability, representing an effective and safe adjunct lipid-lowering therapy.

## COMPARATION BETWEEN THE HOMEOSTATIC MODEL ASSESSMENT FOR INSULIN RESISTANCE AND THE TRIGLYCERIDE-GLUCOSE INDEX: ASSESSING THEIR RELATIOSHIP WITH METABOLIC HEALTH AND NUTRITIONAL FACTORS

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**Introduction.** Insulin resistance is a risk factor for cardiovascular disease. The gold standard to measure insulin resistance is the hyperinsulinaemic-euglycaemic clamp, but this is an invasive and difficult to apply technique. Thus, different surrogate indexes for insulin resistance has been proposed, including the homeostatic model assessment for insulin resistance and the triglyceride-glucose index. The latter not only is useful to assess insulin resistance, but it is also associated with cardiovascular death.
**Aim.** Evaluate how the homeostatic model assessment for insulin resistance and the triglyceride- glucose index compare with regards to their relationship with metabolic health and nutritional factors.
**Methods.** Data from 438 subjects aged 55-80±years were taken into account for this study. Participants fasting metabolic profile was assessed along with anthropometric measurements and the assessment of their dietary habits and physical fitness.
**Results.** As expected there was a positive between both indexes of insulin resistance and anthropometric parameters: body mass index, waist circumference, fat mass; systolic and diastolic blood pressure; and inflammatory markers: interleukin-18 and C-reactive protein. Instead, the triglyceride- glucose index correlated negatively with fat free mass and UKK (Urho Kaleka Kekkonen) fitness index. However, while there were no correlation between the triglyceride- glucose index and nutritional composition of the diet, was not associated with diet parameters instead, the homeostatic model assessment for insulin resistance correlated positively with cholesterol intake, daily net endogenous acid production and omega 6/omega 3 ratio intake and negatively with fibre intake, docosahexaenoic acid, monounsaturated/ as well as omega-3/saturated fatty acids rations and Mediterranean Diet adherence.
**Conclusions.** The homeostatic model assessment for insulin resistance (HOMA-IR) and the triglyceride- glucose index showed a negative association risk factors for cardiovascular disease. However, only the homeostatic model assessment for insulin resistance has a relationship with nutritional parameters.

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## DIFFERENT DISTRIBUTION OF LDL-CHOLESTEROL AND HDL-CHOLESTEROL SUBFRACTIONS IN DYSLIPIDEMIC SUBJECTS WITH CHRONIC KIDNEY DISEASE AND CONTROLS

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**Background.** Chronic kidney disease (CKD) is often associated with increased cardiovascular risk that may be dependent on an increase in triglyceride levels and a decrease HDL-cholesterol (HDL-C) (1). The decrease of the latter is mainly due to the down-regulation of hepatic apolipoprotein A-I (Apo-A1) gene expression. Additionally, the decrease in hepatic lecithin cholesterol acyltransferase (LCAT) mRNA expression may explain a decrease in HDL functionality (2). While it is known that small-dense LDL are a risk factor for cardiovascular disease (CVD), the clinical significance of HDL-C distribution remains to be fully elucidated. OBJECTIVEThe aim of this study was to compare the distribution of cholesterol in the subfractions of HDL and LDL lipoproteins between patients with CKD and controls.
**Methods.** Two hundred and four subjects were recruited for this study and divided into 3 groups: 26 controls with LDL-C goals achieved, 84 controls with LDL-C goals non-achieved and 94 CKD patients with LDL-C goals non-achieved. CKD subjects had estimated glomerular filtration rate values between 30 and 89 ml/min/1,73 $m^2$ with associated dyslipidemia, defined as triglycerides (TG) ≥150 mg/dl or/and HDL-C <50 mg/dl in women and <40 mg/dl in men. The cholesterol distribution in the subfractions of HDL and LDL lipoproteins was determined by Lipoprint system. Differences between the groups were assessed by one-way ANOVA followed by Bonferroni post hoc test or corresponding non-parametric test.
**Results.** Subjects with CKD presented higher levels of total cholesterol, LDL-C and lower of HDL-C. They also presented a higher percentage of cholesterol in small-dense LDL and in small HDL subfractions compared to controls. They also displayed a lower percentage of cholesterol in large-buoyant LDL-C and in large HDL subfractions compared to the two controls groups. No differences in terms of LDL size and HDL-C distribution were detected between controls. The mean size of LDL particles was also lower in patient with CKD.
**Conclusion.** Regardless of the achievement of LDL-C goals, the distributions of LDL-C and HDL-C subfractions and the LDL size were similar in the two control groups. Patients with CKD presented different cholesterol distribution in the LDL and HDL subfractions compared to the control groups. Small-dense LDL contribute to higher cardiovascular risk regardless of LDL-C goals achievement. However, this remains to be clarified for small HDL-C subfractions.

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## DIFFERENT OPERATIONAL DEFINITIONS OF POLYPHARMACY AND THEIR ASSOCIATION WITH THE RISK OF ALL-CAUSE HOSPITALIZATION: A CONCEPTUAL FRAMEWORK USING ADMINISTRATIVE DATABASES

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**Background.** As in all pharmacoepidemiology studies, also in the cardiovascular field it is essential to take into account the clinical complexity of patients, which is very frequently estimated with polypharmacy. We aimed at describing the current heterogeneity of polypharmacy definition, and assessing the association of polypharmacy with clinical outcomes.
**Methods.** Using administrative databases of the local health unit of Bergamo (Lombardy), all subjects aged ≥40 years with at least one reimbursed drug prescription during the year 2017 were identified. We selected from literature relevant operational definitions of polypharmacy. First, we applied World Health Organization (WHO) definition (at least ≥5 different medications, ATC 4th level code). Second, we excluded drug prescriptions associated with short-term treatment. Third, we considered only the prescriptions of drugs with a total annual defined daily doses (DDDs) ≥60. All the approaches were evaluated within one year, one quarter, and one month. A multivariate logistic regression model was performed to estimate odds ratios (OR) and 95% confidence intervals [95% CI] for the association between polypharmacy and the risk of hospitalization for all-causes.
**Results.** Overall, 431,620 subjects were included in our cohort. The DDD-based definition led to estimates with little variability depending on the time windows (range 20.47%-21.16%), while the WHO definition determined the greatest variability (range 39.98%-31.24%). The DDD-based definition identified an older (mean age [SD], 72.6 [10.9]) and more complex cohort of patients (average number [SD] of previous hospitalizations 1.2 [1.7], average number of dispensed drugs 9.7 [3.5]). A dose-dependent increase in risk was observed as the number of the dispensed drugs increases regardless of definitions.
**Conclusions.** Different definitions of polypharmacy led to different prevalence estimates. All definitions showed a dose-dependent association with hospitalization risk, with the definition based on DDDs being the least heterogeneous. However, only a patient-by-patient approach can determine whether or not polypharmacy is appropriate.

## LIPOPROTEIN(A) AND CD34 CIRCULATING CELLS IN HIGHLY EFFECTIVE LIPID-LOWERING STRATEGY

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Lipoprotein(a) (Lp(a) is a macromolecular complex composed of one molecule of an ApoB-100 containing LDL-particle and one molecule of a large highly polymorphic glycoprotein named apo(a). Its contribution to cardiovascular risk has been raising increasing attention. Circulating CD34+ progenitor cells (CD34+CPCs) are known for their tissue regeneration activity through endothelial turnover and angiogenesis. Dyslipidemic subjects seems to have lower CD34+CPCs count; this is however affected by lipid-lowering therapy. Aim of the study was to evaluate Lp(a) plasma levels and CD34+CPCs in a population of heterozygous familial hypercholesterolemia (HeFH) patients at the time of diagnosis and after lipid lowering strategy optimization with triple therapy. We selected from our database patients with available baseline complete lipid panel, Lp(a) plasma levels, CD34+CPCs count, and a follow-up visit after least 12 months where the same parameters had been recorded. Optimized therapy was considered when patient was assuming the maximum tolerated dose of high-efficacy statin, ezetimibe, and an iPCSK9 for at least three months. Patients with prior atherosclerotic cardiovascular disease (ASCVD), hypertension, or with CV comorbidities were excluded from the study. Twenty-six patients met the inclusion criteria (15 males and 11 females). Two patients dropped-out from the study because of lack of compliance. Median age at baseline was 51.5 (IQR 19 years). Median baseline LDL-C levels were 327.5 mg/dl(IQR 103); Lp(a) levels were 56.5 mg/dl (IQR 171). Median CD34+CPCs count was 1.29 (IQR 3.32) cells/µL. After optimized therapy (T1) an 83.4% reduction in LDL-C levels (median 54.3,IQR 64.4) and a 36.2% reduction in Lp(a) levels (median 36,IQR 32) was observed. CD34+CPCs count at T1 was 2.34(IQR 1.4) cells/µL.Interestingly, correlation analysis suggested a correlation between ΔLp(a) and ΔPWV (rho 0.733, $p<0.001$). In conclusion, optimized lipid-lowering triple therapy determined a decrease in Lp(a) plasma levels and a favorable effect on CD34+CPCs count and, likely, on vascular homeostasis.

## SATURATED FATTY ACIDS, BUT NOT TOTAL LIPID INTAKE, PREDICT HDL-CHOLESTEROL SUBFRACTION DISTRIBUTION

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**Background.** Diet is a key discriminant in shaping cardiometabolic health, with both its energy density and nutritional composition being pivotal in dictating its impact on cardiovascular risk (1). In this regard, it is well established that diet is able to impact upon cardiovascular risk by modulating the circulating lipid profile in terms of triglycerides as well as LDL and HDL-cholesterol (2). The impact of the latter on cardiovascular risk appears to be influenced by its distribution in subfractions (3). Nevertheless, whether diet quality is associated with changes in HDL-cholesterol subfraction profile remains to be elucidated. Thus, the aim of this study is to dissect the relationship between diet, both in terms of energy density and nutritional quality, and the subfraction distribution of HDL-cholesterol.
**Participants and Methods.** Thirty seven fertile women aged 41-56 years were included into this study. The study cohort was characterised anthropometrically via the assessment of the body mass index as well as body composition using dual energy X-ray absorptiometry. The metabolic profile of study participants was a ssessed on fasting blood samples by quantifying circulating glucose levels, insulinemia, triglycerides as well as total, LDL and HDL-cholesterol. The latter was further characterised using the Lipoprint System which allows the separation of HDL-cholesterol in ten subfractions categorised in large, medium and small HDL-cholesterol subfractions. Finally, nutrient intake and energy density of the diet was investigated by 24-hour recalls. Correlation analyses were performed using Pearson or Spearman test respectively for normally or not normally distribute variables whereas the predictive power of nutritional factors for HDL-cholesterol distribution was investigated by stepwise linear regression.
**Results.** No relationship was observed between HDL-cholesterol distribution and total energy intake. However, rather than energy per se, the impact of diet on HDL-cholesterol distribution appeared to be related to diet quality. In keeping with this, while carbohydrate and protein intake did not correlate with HDL-cholesterol distribution (both $p>0.05$), lipids correlated negatively with the large ($p=0.008$) and positively with the small ($p=0.042$) HDL-cholesterol subfractions with this relationship being fatty acid specific. Indeed, to the same extent as total lipid intake, saturated fatty acids correlated negatively with cholesterol distribution in the large ($p=0.005$) and positively in the small ($p=0.014$) HDL subfractions. However, this relationship was absent when considering cholesterol, mono and polyunsaturated fatty acid intake (all $p>0.05$). Finally, and most importantly, the intake of saturated fatty acids was also able to predict the levels of small HDL-cholesterol subfractions ($p=0.008$).
**Conclusions.** Considering HDL-cholesterol subfractions being related with dietary factors known to the implicated in increasing cardiovascular risk, particularly saturated fatty acids, HDL-cholesterol distribution may represent a further player in dictating the relationship between diet and cardiovascular health.

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## EFFECTS OF HIGH GLUCOSE ON PCSK9 EXPRESSION IN AORTIC VASCULAR SMOOTH MUSCLE FROM INSULIN-SENSITIVE AND INSULIN-RESISTANT ZUCKER RAT: ROLE OF PCSK9 INHIBITORS.

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**Background and Aims.** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is deeply involved in cholesterol homeostasis and associated to atherogenic processes including vascular smooth muscle cells (VSMCs) dysfunction. Aim of this study was to evaluate in VSMCs from insulin-sensitive (IS) and insulin-resistant (IR) animal models:
1) the basal PCSK9 expression;
2) the expression of PCSK9 under normal glucose (NG) or HG conditions, and
3) the influence of PCSK9 inhibitors on the above HG effects.
**Methods.** Cultured rat aortic VSMC from lean IS and obese IR Zucker rats incubated for 24 hours with 5-25 mmol/L D-Glucose with or without the PCSK9 mAbs alirocumab or evolocumab (40 µg/mL) or the synthetic PCSK9-binding peptide PEP2-8 (10 µm/L) and PCSK9 expression (Western Blot) was measured.
**Results.** In IS-VSMCs: HG increased PCSK9 expression ($n=6$, $p<0.0001$) with effects reduced by alirocumab ($n=6$, $p<0.001$), evolocumab ($n=6$, $p<0.005$), and PEP2-8 ($n=6$, $p<0.01$); IR-VSMC, if compared with IS-ones, showed a basal PCSK9 overexpression ($n=12$,$p<0.0001$) not furtherly influenced by HG ($n=8$, ns) but decreased by alirocumab or evolocumab ($p<0.001$ and $p<0.02$, respectively). The activation of phosphatidylinositol 3-kinases/Akt and mitogen-activated protein kinase/Erk-1/2 pathways are involved in the HG-glucose induced effects on PCSK9 expression.
**Conclusions.** Collectively, these findings indicate that in IS state the exposure to HG may impair VSMC response also through PCSK9-dependent mechanisms that can be improved by both external or internal PCSK9 inhibition. In IR state, VSMCs show a basal overexpression of PCSK9, not furtherly influenced by HG and, at least partially, corrected by PCSK9-inhibitors.

## THE MODULATION OF MITOCHONDRIAL GENES BY PHYSICAL INACTIVITY IN THE ADIPOSE TISSUE CORRELATES WITH PATHOGENETIC FACTORS FOR ATHEROSCLEROSIS: FOCUS ON INFLAMMATORY MARKERS AND NONESTERIFIED FATTY ACIDS

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**Introduction.** Physical inactivity is associated with an increased cardiovascular mortality, even in normal weight subjects (1). In this regard, systemic inflammation and impaired lipid metabolism may be pivotal in linking physical inactivity with cardiovascular disease (2). In particular, interleukin-18 (IL-18) (3) and increased circulating levels of non-esterified fatty acids (NEFA) (4) represent key factors in the pathogenesis of atherosclerosis. Mitochondria have been reported to be at the nexus between inflammation and lipid metabolism, with physical activity playing a key role in modulating mitochondrial biogenesis and function (5). However, the relationship between physical inactivity, adipose tissue mitochondria, inflammation and lipid metabolism remains to be elucidated. Thus, the aim of this study is to determine the effect of bed rest, as a tool to mimic physical inactivity, on mitochondrial gene expression and its relationship with metabolic and inflammatory alterations typical of a sedentary lifestyle.
**Methods.** Eleven male subjects (age: 53±16 years) underwent fourteen days of forced bed rest (BR14). Fasting blood samples were collected at baseline (BR0) and at BR14 in order to assess metabolic (glucose, insulin, lipid profiles and NEFA) and inflammatory markers (TNF-α, IL-18, CRP). Furthermore, biopsies from abdominal and gluteal adipose tissue were collected at BR0 and BR14. Transcriptomic was used to assess the expression of mitochondrial genes in the adipose tissue. Differences between BR0 and BR14 were assessed using Student's t-test whereas the relationship between changes in mitochondrial gene expression from baseline and IL-18 as well as NEFA was investigated using Spearman correlation test. RESULTSBR determined a reduction in total cholesterol and triglycerides, an increase in circulating glucose levels along with increased inflammatory markers. Among the over 200 probed mitochondrial genes, seventeen displayed a significant modulation after BR14. These genes were involved in the Krebs cycle (downregulation of OGDH), beta-oxidation (downregulation of ACADS, ACAD10) or electron transport chain proteins as complex I (downregulation of NDUFS2, upregulation of NDUFA1, NDUFB2, NDUFB3), complex III (upregulation of CYB5R4 and UQCRB), complex IV (upregulation of COX6C, COX7A2, COX7B, COX7C, COX8A), ATP synthase (upregulation of ATP5H, ATP5G2) and downregulation of COQ4. Circulating levels of inflammatory markers and NEFA were significantly correlated to changes in mitochondrial gene expression. In particular, NEFA levels negatively correlated with gluteal tissue expression of UQCRB (p=0.036), COX6C (p=0.003), COX7C (p=0.003), COX8A (p=0.007) and NDUFB3 (p=0.023). Serum TNF-α was positively associated with gluteal adipose tissue expression of COQ4 (p=0.014), and OGDH (p=0.014). With regard to the abdominal adipose tissue gene expression, serum IL-18 was negatively correlated with COQ4 (p=0.021) and CRP negatively with CYB5R4 (p=0.028).
**Conclusions.** Physical inactivity induces alterations in adipose tissue mitochondrial gene expression associated with circulating inflammatory and lipid biomarkers related to the development of atherosclerosis.

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## ANGIOPOIETIN-LIKE 3 KNOCK-OUT MOUSE MODEL PROFILING UNDER FASTING AND FED CONDITIONS

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**Aim.** Angiopoietin-like 3 (ANGPTL3) is a protein that controls lipids and lipoproteins metabolism through lipoprotein lipase and endothelial lipase inhibition and thus preventing lipoprotein-derived triglycerides hydrolyzation. Here we present the metabolic profile of ANGPTL3 deficient mice in their basal state and during the development of metabolic disorders under fasting and fed conditions.
**Methods.** Angptl3 KO mice (C57BL6/J background) and their littermate controls (wild-type, WT) were fed a chow and a High Fat Diet (HFD, 60% kcal from lipids) for 16 weeks. During the experimental protocol, the metabolic phenotype was assessed, along with changes in plasma lipids under fast, fed, and fast-refeed settings; the profile of lipids absorption was assessed with an Oral Lipid Tolerance Test (OLTT).
**Results.** ANGPTL3 KO mice fed ad libitum a chow diet are hypolipidemic (plasma triglycerides levels: 42,42±8,80 mg/dL in ANGPTL3 KO mice compared to 122,02±55,09 mg/dL in WT mice; plasma cholesterol levels: 44,00±9,11 mg/dL in ANGPTL3 KO mice compared to 76,51±15,87 mg/dL in WT mice). The OLTT on chow-fed mice after 16h of fasting suggests that KO mice have lower lipid levels at all time points. Moreover, on HFD, ANGPTL3 KO mice gain less body weight, suggesting a metabolic advantage against dysmetabolism development compared to WT animals.
**Conclusions.** This preliminary profiling of ANGPTL3 KO mice under fasting and fed conditions as well as on chow diet or HFD highlights the hypolipidemia of these models, and the potentially beneficial impact of ANGPTL3 deficiency in their metabolism compared to controls.

## EFFECTS OF LIFESTYLE ADVICE COUPLED WITH A BEETROOT-BASED NUTRACEUTICAL ON 24-HOUR BLOOD PRESSURE IN A POPULATION WITH HIGH-NORMAL BLOOD PRESSURE OR GRADE 1 HYPERTENSION AND LOW CARDIOVASCULAR RISK: A PILOT STUDY

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**Background.** Lifestyle changes are recommended for high-normal blood pressure (BP) or grade 1 hypertension with low-moderate cardiovascular (CV) risk, eventually associated with a nutraceutical. Among nutraceuticals available, most with unproven or even unlikely benefits, bioactive substances enriched with nitrates (i.e. red beets extracts) might have vasodilator effects and may decrease BP. We evaluated the effect of ESC/ESH guideline lifestyle advice together with a beetroot-based nutraceutical, on 24-hour BP in a population with high-normal office BP and low-moderate CV risk as a pilot study in two ESH Excellence Hypertension Centres.
**Methods.** A longitudinal observational study was conducted on 43 consecutive subjects referred to two hypertension centres in Italy. Twenty-four-hour ambulatory BP monitoring (ABPM) were carried out at baseline and three months after written but generic advice for lifestyle changes associated with a nutraceutical containing 500 mg of dry beetroot extract.
**Results.** Mean age 50±11 years, 54% males, 58% overweight/obese, mean office BP 135±3/85±3 mmHg. At baseline, 24-hour BP, daytime BP and night-time BP were 127±7/80±6 mmHg, 131±8/83±6 mmHg and 118±8/70±5 mmHg, respectively. After a median follow-up of 98 (92-121) days, all BPs, except night-time diastolic BP, were significantly decreased: -3±6/-2±4 mmHg for 24-hour BP, -3.9±6.0/-3.0±4.0 mmHg for daytime BP and -3.3±7.4/-1.3±4.7 mmHg for night-time BP, respectively. BP decreased independently of baseline BP level, gender, smoking status and BMI, while a greater decrease in BP was observed in older patients.
**Conclusions.** Our pilot study shows that written but generic, not personalized, lifestyle advice coupled with a beetroot-based nutraceutical, a source of organic nitrates, may be a valid initial non-pharmacological approach in subjects with high-normal BP or grade 1 hypertension and low-moderate CV risk.

## SCORE2 ESTIMATED WITH 24-HOURS BLOOD PRESSURE AND LDL-C EQUATIONS ON CARDIOVASCULAR RISK AND CONTROL IN A POPULATION EVALUATED FOR HYPERTENSION

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**Introduction.** The 2021 ESC Guidelines on Cardiovascular Disease Prevention were based on the SCORE2 /SCORE2-OP cardiovascular risk (CVR) assessment model. We assessed the impact of SCORE2/SCORE2-OP on CVR stratification and prevalence of low-density lipoprotein cholesterol (LDL-C) control after calculating SCORE2 using office (OBP) and ambulatory blood pressure (ABPM) and LDL-C using three validated equations in a hypertensive population.
**Methods.** A cross-sectional study on 1539 consecutive patients with valid ABPM. LDL-C was calculated using the Friedewald formula (F), its modification by Martin (M), and the Sampson (S) equation. SCORE2/SCORE2-OP was estimated using OBP, mean systolic daytime (+5 mmHg adjustment), and 24-h mean systolic blood pressure (+10 mmHg adjustment). Individual CVR by 2021 ESC Guidelines (and SCORE2/SCORE2-OP) was compared to the 2019 ESC/EAS Guidelines (and SCORE).
**Results.** Mean age 60±12 years, male prevalence (54%). Mean LDL-C values were 118±38 mg/dL (F), 119±37 mg/dL (M), and 120±38 mg/dL (S). No differences emerged comparing the average SCORE2 calculated with OBP (6% IQR 3-10), 24h ABPM (7% IQR 4-11), and daytime SBP (7% IQR 4-11). SCORE2/SCORE2-OP and 2021 ESC Guidelines reclassified the CVR. The low-moderate risk group decreased by 32%, whereas the high and very-high-risk groups increased by 18% and 12%, respectively. We found a significant reduction in reaching the LDL-C goals regardless of the equation used to calculate it, except for those >65 years, where results were confirmed only by using the M.
**Conclusion.** SCORE2/SCORE2-OP and 2021 ESC Guidelines recommendations led to a non-negligible CVR reclassification and subsequent lack of LDL-C at goal, regardless of estimating SCORE2 using OBP or ABPM. OBP is good enough when correctly carried out, to assess CVR. Calculating the LDL-C with the M may be the best choice in specific settings.

## PULSE WAVE VELOCITY IMPROVEMENT AND LIPOPROTEIN(A) CHANGE IN HIGH EFFECTIVE TRIPLE LIPID-LOWERING THERAPY

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Evidence that elevated lipoprotein(a) (Lp(a)) levels contribute to cardiovascular disease (CVD) is emerging. Pulse wave velocity (PWV) has also emerged as a non-invasive, valid, and reliable measure of arterial stiffness. Its role as a marker of mechanical vascular impairment and its association with coronary, cerebral, and carotid atherosclerosis has been established.
Aim of the study was to evaluate Lp(a) plasma levels and PWV values in a population of heterozygous familial hypercholesterolemia (HeFH) patients at the time of diagnosis and after lipid-lowering strategy optimization (high efficacy statin plus ezetimibe plus PCSK9-inhibitor). Eligible subjects were selected from our database. Exclusion criteria included other cardiovascular comorbidities – i.e., prior atherosclerotic cardiovascular disease (ASCVD), treated and untreated hypertension and diabetes. Baseline data (T0) included treatment-naïve complete lipid panel, Lp(a) plasma levels and PWV values. Optimized therapy – i.e.,maximum tolerated dose of rosuvastatin or atorvastatin plus ezetimibe plus evolocumab or alirocumab – was then prescribed; compliance to such treatments was assessed every three months. A 12-month follow-up visit was scheduled (T1) and the same parameters were recorded.
Twenty-six patients met the inclusion criteria (15 males,11 females). Two patients dropped-out from the study because of poor compliance to treatment. Median age at baseline was 51.5 (IQR 19) years. Median baseline LDL-C levels were 327.5 mg/dl (IQR 103); Lp(a) levels were 56.5 (IQR 171) mg/dl. Median baseline PWV was 14.1 (IQR 4.9) m/sec. After optimized therapy (T1) LDL-C levels dropped to 54.3 (IQR 64.4,-83.4%); plasma Lp(a) dropped to 36 (IQR 132,-36.2%) mg/dl. A reduction in median PWV values was also registered (median 9.37,IQR 1.32,-33.5%). Interestingly, correlation analysis suggested an inverse correlation between ΔLp(a) and ΔPWV (rho -0.633,p=0.001).
In conclusion, the study suggests a correlation between an increased atheromasic burden-as expressed by higher Lp(a) baseline values-and smaller change in arterial stiffness as expressed by PWV values, despite the larger reduction in Lp(a) levels after optimized triple lipid-lowering therapy.

## A NONINVASIVE EVALUATION OF LIVER FIBROSIS RISK IN SUBJECT WITH SEVERE HYPERTRIGLYCERIDEMIA: A SINGLE CENTER COMPARISON BETWEEN MCS AND FCS

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**Background and Aim.** Lipoprotein lipase (LPL) is the crucial enzyme responsible for (TG) hydrolysis in chylomicrons and very-low density lipoproteins (VLDL). In patients with metabolic features, the dysfunction of this pathway can lead to the development of chylomicronemia (TGs>1000 mg/dl, known as multifactorial chylomicronemia or MCS). Non-alcoholic fatty liver disease (NAFLD) is frequent in patients with features of the metabolic syndrome (MetS), obesity, or type 2 diabetes and associates with an increased risk of liver fibrosis. Rarely, chylomicronemia is characterized by LPL deficiency (LPLD), an autosomal recessive disease called familial chylomicronemia syndrome (FCS), which is associated with an increased risk of recurrent pancreatitis and hepatosplenomegaly. The hepatic consequences of FCS and MCS has been only partially investigated. This study aims to compare liver stiffness and the risk of liver fibrosis between FCS and MCS.
**Methods.** To this aim, 83 patients with chylomicronemia were consecutively enrolled in the LIPIGEN study from the lipid clinic of the Policlinic Umberto I, Sapienza University of Rome. All patients underwent molecular diagnosis of FCS or MCS. Demographic, clinical and biochemical data were retrospectively revised by revising medical charts from enrollment (last visit) backward to baseline (first visit in the lipid clinic). Biochemical parameters collected were used to non-invasively estimate liver fibrosis by using FIB-4 index. At last visit all participants underwent routine liver ultrasonography and stiffness measurement after at least 4-h fast using transient elastography (Fibroscan and/or acoustic radiation force impulse (ARFI)).
**Results.** In this cohort 22 patients were classified as FCS and 61 as MCS. The median age at enrolment was 50.5 years (IQR 40.5-66.5) in FCS and 47.2 years (IQR 37.0 – 57.0) in MCS (P=0,33). The two cohort were equally distributed between sexes and no differences were found in the baseline triglycerides levels. The median follow-up during in FCS was significantly longer than in MCS respectively 7.7 (IQR 3,37 – 11,33). years vs 4.0 (IQR 0,91- 6,91). As expected, MCS were showing a greater prevalence of metabolic features and a better response to triglycerides lowering therapies; in fact, the median on treatment TG levels was significantly higher in FCS than in MCS (1293 mg/dl vs 818 mg/dl; p=0.002). At last visit no difference were found in the prevalence of NAFLD between the two cohorts (84.8% of MCS and 90.5% of FCS patients, P=NS). Despite being within the normal range, results showed that FCS patients were showing a greater hepatic stiffness when compared to MCS group (5.73 kPa vs 3.89 kPa, respectively, P=0.03). The estimation of Fib-4 at last visit showed similar results with FCS showing an higher values as compared to MCS (1.84 vs 1.0, respectively, P=0.004).Conclusion: In this study, NAFLD was commonly observed in both FCS and MCS subject. Nevertheless, our results showed that liver fibrosis might be more severe in FCS and MCS; this observation may be the results of the liver exposure to very high triglyceride levels since birth in the monogenic chylomicronemia and potentially suggest the need of a more careful assessment of liver disease in these patients. Further study in larger cohort are needed to definite clarify if liver fibrosis might be considered another hallmark of FCS.

## HIGH DENSITY LIPOPROTEIN ANTI-INFLAMMATORY CAPACITY IS INCREASED IN SUBJECTS WITH LOW LARGE PARTICLE DIMENSION

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High density lipoprotein (HDL) particle size has been identified as a strong determinant of cardiovascular (CV) risk, with the amount and number of large HDL particles being inversely related to CV risk. Previous studies hypothesized that HDL subspecies might be involved in different functions, therefore differentially impacting the CV risk. One of the most studied properties of HDL is its anti-inflammatory capacity (AIC). However, little is known about the relationship between HDL particle size and AIC. We aimed to unveil whether and how HDL particle size influenced AIC. To this end, 24 women (45-67 years) were included in this study. Plasma samples underwent Lipoprint analysis to characterize particle size distributions and amounts. According to the distribution of large HDL particles, the population was divided into high large HDL particles (n=12, group 1), and low large HDL particles (n=12, group 2). Plasma samples of the two groups were pooled and subjected to density gradient ultracentrifugation in order to obtain purified HDL2+HDL3 fractions. The purified fractions were assessed for their AIC by evaluating the decrease/increase in VCAM-1 expression in HUVEC cells following TNF-α treatment. Plasma samples were also analyzed for Paraoxonase-1 (PON-1), Glutathione peroxidase 3 (GpX3), total antioxidant capacity (TAC) and Myeloperoxidase (MPO). Patients belonging to group 1 had higher PON-1 activity than group 2 ($p<0.001$), whereas TAC, GpX3, MPO activities were unchanged. Interestingly, the AIC was higher in group 2 than group 1. Although it might be considered counterintuitive, a previous work found that lipid poor and small HDL particles produced by the fusion with a discoidal particle made of human apoAI and phospholipids had stronger anti-inflammatory properties. Thus, our results confirm that different particle size have a diverse impact on HDL function, suggesting that the right mix of multiple HDL particle sizes could be optimal to maximize the cardio-protective benefits.

## POSSIBLE LINK BETWEEN AUTOANTIBODY EXPRESSION IN PATIENTS WITH AUTOIMMUNE DISEASES, LIPID PROFILE AND CARDIOVASCULAR RISK

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In patients with rheumatic diseases, there is an increased cardiovascular risk. Therefore the need to investigate mechanisms underlying such increased risk is important and we aim to study a possible influence of dyslipidemic status. Sixty patients, 39 females and 21 males, were examined in this study: 40 with Rheumatoid Arthritis, 8 with SLE, 7 with Systemic Sclerosis and 5 with Sjögren's Syndrome. Median age 59.4 years. LDLc values (obtained by Friedewald's formula) and autoantibody expression(FR, ACPA, ANA, ENA, Ab anti-centromere, Ab anti-Scl70, Ab anti-Ro/SSA, Ab anti-La/SSB, Ab anti-Beta2glycoprotein 1, Ab anti-cardiolipin) of the subjects analyzed were estimated at the first visit and after 3 months and 6 months of therapy for underlying rheumatologic disease. Regarding patients with RA, only 2 patients had MACE in their history (one patient with previous STEMI undergoing revascularization, a second with history of ischemic-based cerebral stroke before age 50 years), while 5 patients were dyslipidemic at baseline and 7patients among all were on Statin therapy (Rosuvastatin 20 mg) and had recently performed an arterial TSA Doppler examination with median IMT about 1.7 mm. Median LDLc was 125mg/dl. At follow-up after 3 months, a 20 % reduction in FR and ACPA expression was documented with mean LDLc estimated to be 115 mg/dl. After 6 months there was a further reduction in FR and ACPA values of about 30%, with mean LDLc of 107 mg/dl and IMT brought into view and remaining stable in Dopplerre-evaluations. No changes had been made to hypolipidemic therapy at follow-up visits. In SLE patients, there were no prior MACE and only 3 patients were on Statin therapy (Torvastatin 20 mg) and Ezetimibe at baseline. Median LDLc at T0 was 119. There were no data on intimal thickness at the level of the supra-aortic trunks in these patients. At 3 and 6 months, an improvement in the trend of LDL values in relation to lower autoantibody production was also observed in this case, in the absence of changes in the therapeutic set-up (ANA with homogeneous pattern descended to 1: 80 from 1: 320 and mean LDLc of 105 mg/dl).Considering subjects with Scleroderma and Sjögren's Syndrome, also without relevant clinical history in history, they showed a good LDL response (median LDLc at baseline of120 mg/dl) associated with the reduction of anti-Scl70 Ab values (by 18%), anti-centromere(by 16%), anti-Ro/SSA (by 17%) and anti-La/SSB (by 16%) with LDLc descended to 104mg/dl. Thus, it can be observed that the lipid profile improves as antibody expression decreases,which highlights how there may be an interrelationship between the inflammatory state and endogenous cholesterol values, that can be further investigated with a view to further molecular markers such as miRNA.

## CHOLESTEROL ESTERIFICATION IS HAMPERED IN ALZHEIMER'S DISEASE AND CHOLESTERYL ESTERS COMPOSITION IS CONSEQUENTLY ALTERED

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Several epidemiological studies indicate a strong inverse association between the risk of developing Alzheimer's disease (AD) and plasma HDL-C levels. The mechanism by which plasma HDL influence the pathogenesis and progression of AD remains unsolved and the crucial step of cholesterol esterification in HDL metabolism could be involved. The purpose of this study was to evaluate cholesterol esterification and cholesteryl esters (CEs) composition in plasma and cerebrospinal fluid (CSF) of AD patients. In this study, 70 AD patients and 74 cognitively-normal controls comparable for age and sex were enrolled. Lipids and lipoprotein profile, cholesterol esterification, and cholesterol efflux capacity (CEC) were evaluated in plasma and CSF using assays set for measurement in plasma, which were appropriately modified for CSF. CEs and phospholipids (PLs) species were evaluated with omics techniques, LC-MS and LC-MS/MS respectively, in plasma and CSF of a subgroup of AD and controls. AD patients have normal plasma lipids, but significantly reduced unesterified cholesterol (UC) and unesterified/total cholesterol ratio (UC/TC). Lecithin: cholesterol acyltransferase (LCAT) activity and cholesterol esterification rate (CER), two measures of the efficiency of the esterification process, were reduced by 29% and 16%, respectively, in plasma of AD patients. Plasma HDL subclass distribution in AD patients was comparable to that of controls, but the content of small discoidal preβ-HDL particles was significantly reduced. In agreement with the reduced preβ-HDL particles, CEC mediated by the transporters ABCA1 and ABCG1 was reduced in AD patients' plasma. The CSF UC/TC was increased in AD patients, and CSF CER and CEC from astrocytes were significantly reduced in AD patients. In the AD group, a significant positive correlation was observed between plasma UC and UC/TC ratio with Aβ1-42 CSF content. Cholesteryl esters composition in CSF of AD revealed significant enrichment in saturated fatty acids (FA) and monounsaturated FA and a depletion in polyunsaturated FA in cholesterol species compared to controls. Specifically, cholesteryl ester palmitate and oleate were increased ($p=0.001$; $p=0.005$); while linoleate, the major LCAT substrate, was reduced ($p=0.029$) in AD. Plasma samples did not show any difference in CEs composition and CSF PLs species analysis displayed no significant modification. Taken together these data indicate that cholesterol esterification is hampered in plasma and CSF of AD patients and that plasma cholesterol esterification biomarkers (UC and UC/TC ratio) are significantly associated with disease biomarkers (i.e., CSF Aβ1-42). CEs composition confirmed the crucial role of cholesterol esterification in AD, providing new insights and possibilities for pharmacological targeting.

## ASSOCIATION OF CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY AND STRESS ECHOCARDIOGRAPHY WITH LONG-TERM CARDIAC OUTCOME: A COMPARISON STUDY

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**Aims.** In the challenging clinical scenario of suspected chronic coronary syndrome (CCS) there are no studies that compared the long-term prognostic value of coronary computed tomography angiography (CTA) with stress echocardiography (SE). Furthermore, no data are currently available regarding the association of such diagnostic tests with outcome when both tests were performed in the same cohort of patients. This study aimed to assess which variables on CTA and vasodilator SE are best associated with long-term cardiac outcome in patients presenting for suspected CCS who performed both tests.
**Methods.** We identified 397 patients with suspected CCS who, between 2007 and 2019, underwent both SE and CTA within 30 days. Coronary artery calcium score (CACS) and the number of coronary arteries with diameter stenosis >50% were assessed on CTA. The presence of reversible regional wall motion abnormalities (RWMA) and reduced Doppler coronary flow velocity reserve in the left-anterior descending coronary artery (CFVR) were assessed on SE. The association of SE and CTA variables with cardiac outcome (cardiac death or myocardial infarction) was evaluated using Fine and Gray competing risk models.
**Results.** During a median follow-up of 10 years, 38 (9.6%) patients experienced a nonfatal myocardial infarction and 19 (4.8%) died from a cardiac cause. RWMA (HR 7.189, $p<0.001$) and a lower CFVR (HR 0.034, $p<0.001$) on SE, along with CACS (HR 1.004, $p<0.001$) and the number of >50% stenosed coronary vessels (HR 1.975, $p<0.001$) on CTA, were each associated with cardiac events. After adjusting for covariates, only CACS and CFVR remained associated (both $p < 0.001$) with cardiac outcome.
**Conclusion.** Our data suggest that only CFVR on vasodilatory SE and CACS on CTA are independently and strongly associated with long-term cardiac outcome, unlike RWMA or the number of stenosed coronary arteries, usually considered the hallmarks of coronary artery disease on each test.

## CORONARY INFLAMMATION ON CHEST COMPUTED TOMOGRAPHY AND COVID-19 MORTALITY

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**Aims.** The main factors associated with coronavirus disease-19 (COVID-19) mortality are age, comorbidities, pattern of inflammatory response and SARS-CoV-2 lineage involved in infection. However, the clinical course of the disease is extremely heterogeneous, and reliable biomarkers predicting adverse prognosis are lacking. Our aim was to elucidate the prognostic role of a novel marker of coronary artery disease inflammation, peri-coronary adipose tissue attenuation (PCAT), available from high-resolution chest computed tomography (HRCT), in COVID-19 patients with severe disease requiring hospitalization.
**Methods.** Two distinct groups of patients, admitted to Parma University Hospital in Italy with COVID-19 in March 2020 and March 2021 (first and third wave peaks of COVID-19 pandemic in Italy, with prevalence of wild-type and B.1.1.7 SARS-CoV-2 lineage, respectively) were retrospectively enrolled. The primary endpoint was in-hospital mortality. Demographic, clinical, laboratory, HRCT data and coronary artery HRCT features (coronary calcium score and PCAT attenuation) were collected to establish which variables were associated with mortality.
**Results.** Among the 769 patients enrolled, 555 (72%) were discharged alive and 214 (28%) died. In multivariable logistic regression analysis age ($p<0.001$), number of chronic illnesses ($p<0.001$), smoking habit ($p=0.006$), P/F ratio ($p=0.001$), platelet count ($p=0.002$), blood creatinine ($p<0.001$), non-invasive mechanical ventilation ($p<0.001$), HRCT visual score ($p<0.001$) and PCAT ($p<0.001$) were independently associated with in-hospital mortality.
**Conclusion.** Coronary inflammation, measured with PCAT on HRCT, was independently associated with higher mortality in patients with severe COVID-19.

## PROBING THE EFFICACY OF NATURAL AND NOVEL SYNTHETIC PCSK9 INHIBITORS IN REDUCING NEUROINLFAMMATION, Aβ-INDUCED NEUROTOXICITY AND CHOLESTEROL UPTAKE IN HUMAN CEREBRAL CELL MODELS

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Alteration of cerebral cholesterol homeostasis together with neuroinflammation are mechanisms leading to Alzheimer's disease (AD). The proprotein convertase subtilisin/kexin 9 (PCSK9), other than regulating plasma cholesterol, is expressed also in the central nervous system (CNS), where it worsens the β-amyloid (Aβ)-induced neurotoxicity and reduces neuronal cholesterol uptake, suggesting an involvement in neurodegeneration. This in vitro study aims to prove the putative role of PCSK9 on neuroinflammation and to investigate the potential protective effect of its pharmacological inhibition with natural and newly-synthesized molecules. Preliminary studies on inflammation were performed on astrocytoma cells (U373) exposed to recombinant PCSK9 and Aβ-fibrils evaluating the expression of inflammatory genes (qRT-PCR) and cytokines' release (ELISA assay). Both natural (magnoflorine, a berberine analogue) and newly-synthesized molecules (MR compounds) with previously proven PCSK9 inhibitory activity were tested on human microglial cells (HMC3) and human neuroblastoma cells (SH-SY5Y) to assess their potential protective effect on Aβ-induced neurotoxicity (MTT assay), neuronal cholesterol uptake (radioisotopic techniques) and neuroinflammatory cytokines' secretion (ELISA assay). PCSK9 significantly enhances the Aβ-induced neuroinflammatory response, by increasing IL-6, IL-1β, TNF-α and inflammasome-related (Pyrin, NLRP3, NLRC4) gene expression, as well as MCP1 release ($p<0.05$). Microglial viability, significantly reduced after incubation with Aβ-fibrils (-28%; $p<0.0001$), was dose-dependently restored by synthetic PCSK9 inhibitors, with the most evident effect for MR-533 at 10μM ($p>0.05$ vs basal condition), as well as by magnoflorine at 200μg/ml ($p>0.05$ vs basal condition). Neuronal cholesterol uptake, reduced when PCSK9 is overexpressed ($p<0.05$), is restored after treatment with MR-3 at 10 μM ($p<0.01$). Furthermore, magnoflorine treatment (100μg/ml) in HMC3 cells dampened Aβ-triggered IL-6 release ($p<0.05$).
In conclusion, our in vitro results point to PCSK9 as pivotal in the modulation of neuroinflammation and its pharmacological inhibition reduces Aβ-induced neurotoxicity and restores neuronal cholesterol uptake, potentially opening the way for new approaches in the treatment of AD.

## COMBINED LNCRNAS EVALUATION IN PLAQUES AND PLASMA FROM ATHEROSCLEROTIC PATIENTS HIGHLIGHTED H-19 OVEREXPRESSION AS POTENTIAL BIOMARKER

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**Introduction.** Atherosclerosis is a disease characterized among other causes by the lipoprotein accumulation and the chronic inflammation of the arterial wall triggering the development of atherosclerotic plaques. Even if long non-coding RNAs (lncRNAs) are emerging as regulatory molecules involved in the pathogenesis of an increasing number of diseases, to date, their association with atherosclerosis is still poorly investigated (1). Thus, we aimed to evaluate lncRNAs expression in plaques and plasma to find novel diagnostic and/or therapeutic biomarkers through a comprehensive RNA-Seq analysis followed by an accurate bioinformatic analysis.
**Materials and Methods.** Human carotid atherosclerotic plaques, their adjacent regions (lower grade lesions) and plasma from 15 patients undergoing endarterectomy were collected (44 samples). All samples were processed for RNA-Seq analysis by using Next Generation Sequencing (NGS) protocols; libraries were prepared with the Illumina Stranded Total RNA Prep kit and sequencing was made on Illumina NextSeq550Dx instrument). The bioinformatic analysis was carried out by using different pipelines in R. Quality controls showed that all tissue samples had good quality. Reads from plasma samples were filtered and deduplicated before the following analysis. After mapping and quantification analysis, principal component analysis (PCA), differential expression analysis, pathway enrichment analysis and network analysis were carried out. Additionally, we compared our results against data of atherosclerotic and non-atherosclerotic arteries from Genotype-Tissue Expression (GTEx) Project database, that contain transcriptomic information about 54 tissues sites across 1000 deceased donors.
**Results.** Clustering analysis and PCA showed a clear separation between plaque and adjacent region tissue samples. Differential expression analysis highlighted 915 differentially expressed genes in plaques versus adjacent regions, with 798 over-expressed and 117 under-expressed genes in plaque versus adjacent region samples. Pathway enrichment analysis using KEGG database showed that the over expressed genes in plaque group belong to inflammation and immunity pathways, thus confirming the biological mechanism of atherogenesis. The comparison with GTEx dataset showed that, of the 200 differentially expressed genes in GTEx, 100 were concordant with the differentially expressed genes of our dataset, thus supporting our results. We found 48 lncRNAs differentially expressed and the top five over expressed in plaques versus the adjacent regions showed a log fold change (logFC) higher then 3. Network analysis showed interesting relationships between some lncRNAs and their respective targets that have to be deeper investigated. To analyse data in plasma samples, correlation of expression was made in order to find a good circulating biomarker reflecting the alterations observed in plaques. Higher was the expression of a gene in plaques respect the adjacent regions (gene with higher logFC), higher was the expression in plasma, thus producing a positive correlation. Among all the differentially expressed genes (coding and non-coding), lncRNA H-19 had one of the highest logFC (logFC=4.3; P value=0.000040) and, among the lncRNAs with a positive correlation in plasma, it is the one with the highest and significant positive expression correlation (Correlation value=0.6; P value=0.022). Moreover, GTEx supports this data because lncRNA H-19 is more expressed in atherosclerotic samples in GTEx than in controls.
**Conclusion.** For the first time a high lncRNA H-19 expression was observed in human atherosclerotic carotid plaque tissues with a high and significant correlation of its plasma concentration. Our data together with scattered literature highlighted lncRNA H-19 functional role in triggering atherosclerosis by acting through different pathways.

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## HELPER-DEPENDENT ADENOVIRAL VECTOR EXPRESSING MURINE CHIMERIC PROTEIN AMELIORATES LIPID PROFILE IN LDLR-DEFICIENT MICE

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Individuals diagnosed with familial hypercholesterolemia (FH), a monogenic genetic disorder, suffer from an inability to process low-density lipoprotein (LDL), which leads to its build-up most significantly in the arterial walls apart from other tissues. Therefore, without treatment, these individuals are at a higher risk for developing life-threatening conditions such as severe heart disease, leading to early mortality. To date, several treatments are available but have cons such as side effects, intolerance issues, and more importantly, rely on at least partial presence of endogenous functioning LDLRs to work effectively. To better optimize FH treatment, we have turned to using gene therapy tactics to develop Helper-dependent adenoviral vectors, which carry the murine LDLR/Tf chimeric protein. In vitro and in vivo functionality analysis of the murine chimeric protein produced indicated that the murine fusion protein can effectively correct the phenotype of LDLR deficient cells and can increase LDL uptake. These results are promising for further production and characterization of the human LDLR/Tf chimeric protein and show the potential for additional studies that evaluate the safety of this gene therapy treatment as a long-term correction solution, which prevents activating the immune system by avoiding toxic build-up.

## THE EFFECTS OF DIRECT ORAL ANTICOAGULANTS AND VITAMIN K ANTAGONISTS ON THE SERUM METABOLOMIC AND LIPOPROTEOMIC PROFILES OF ATRIAL FIBRILLATION PATIENTS

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Atrial fibrillation (AF) is the most common cardiac arrhythmia with a clinical relevance observed in medical practice. Long-term oral anticoagulation is the primary therapy for the prevention of ischemic stroke in patients diagnozed with AF. Different types of oral anticoagulant drugs can have specific effects on the metabolism of AF patients. We have characterized, for the first time to the best of our knowledge, the serum metabolomic and lipoproteomic profiles of AF patients treated with two classes of anticoagulants: vitamin K antagonists (AVKs) and direct oral anticoagulants (DOACs).Serum samples of 177 AF patients (median age 78 years, 62% males, 70% on DOACs treatment) were analyzed via high resolution 1H NMR spectroscopy. Data on 25 metabolites and 112 lipoprotein-related fractions were quantified and analyzed with multivariate and univariate statistical approaches. Our data show that patients treated with AVKs and DOACs present significant differences in their profiles: lower levels of alanine and lactate, as well as higher levels of HDL cholesterol, apolipoprotein A1 and 12 HDL cholesterol fractions and subfractions are characteristic of serum profile of patients on DOACs' therapy. Our results support the usefulness of NMR-based metabolomic approach in the AF setting for the description of the pharmacological effects of anticoagulants on patient circulating metabolites and lipoproteins. The higher levels of HDL cholesterol observed in patients treated with DOACs could contribute to explaining their reduced cardiovascular risk and may suggest a potential pleiotropic effect of these drugs. These results suggest the need of further studies in this direction to fully understand possible clinical implications.

## UNVEILING THE LIPID METABOLISM IN MACROPHAGES AND KUPFFER CELLS IN C57BL/6 MICE

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**Background.** Macrophages and Kupffer Cells have a key role in the lipid metabolism and in modulation of the inflammation. However, there are conflicting results about the transcriptomic regulation of these two important processes in mice fed with a rich fat diet.
**Methods.** Single-cell RNA-sequencing of the total aorta population and of CD45+ cells in the liver is performed on C57BL6/J mice, fed for 12 weeks with SD (11% fat) or WD-HFD (60% fat). The data are processed with Seurat pipeline with a 0.1 resolution. The differently expressed genes (DEGs, Padj-value>0.05) are analyzed with DAVID, IPA and EnrichR software. The data are obtained from the published datasets under the GEO: GSE156059 (40,000 cells) and SCP1361 (24,001 cells).
**Results.** The top expressed markers in both KC and macrophages were CD68, C1qb and Lyz2, suggesting for a common metabolic function. Thus, we analyzed the total DEGs (n=450), comparing the macrophages of the aorta for the diet and were enriched in pathways such as coronary disease and inflammatory pathways. The top DEGs were ANGPTL4 (log2FC=0.41), LPL (log2FC= -0.70) and CD36 (log2FC= -0.51). Comparing only the Kupffer cells population for the diet, there were an increase of Angptl4 (log2FC=2.19) that seems to be related to the increased activation of PPARα (log2FC=2.98). The DAVID enrichment analysis shows that PPARα also reduces the response to lipopolysaccharide (FDR=2.86x10E-3) while the upregulated genes (log2FC > 1, n=523) are enriched for the lipid hydroxylation (FDR=9.05x10E-5) and the balanced response of LPL (log2FC=0.699) and CD36 (log2FC=1.2). On the other hand, the prediction analysis in IPA shows a tendency to decrease in IL-12 (z-score=-1.298) and IL-6 (z-score= -1.091) signaling suggesting for an activation of anti-inflammatory pathways.
**Conclusion.** The high fat diet seems to activate the expression of Angptl4 in KC and macrophages whether this expression mediates different metabolic signaling is under investigation.

## CARDIOVASCULAR RISK AWARENESS: WHAT DOES PATIENT FEEL?

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**Introduction.** Greater people's awareness of cardiovascular risk factors could lead to better compliance with behaviour and/or pharmacological therapy. Our aim was to evaluate the cardiovascular risk (CVR) awareness of different groups of patients with cardiovascular risk.
**Materials and Methods.** A total of 284 ambulatory patients were enrolled (from May to July 2023) in this study: 148 (52%) from Hypertension-dyslipidemia Center, 92 (32.3%) from Diabetes Centre and 45 (15.7%) from Obesity Center. All subjects were asked to complete (by their-self) questionnaire to evaluate their awareness of CVR.The questionnaire was composed of 20 questions, which included demographic characteristics, personal and family history of CVD, information needed to calculate CVR according to SCORE parameters. Their perception of CVR was compared with doctors' CV score evaluation.
**Results.** 32.1% of patients had low CVR, 26% moderate CVR, 14.6% high CVR and 27.3% very high CVR.Only 40% of patients had a correct perception of their CVR, among patients with incorrect CVR perception, the majority of patients underestimated their risk (85%) and only 15% overestimated it. In particular, CVR was underestimated by 54.7% of patients of Hypertension-dyslipidemia Center, by 49.2% of Diabetes Center and by 41% of Obesity Center. A significant association was found between CVR overestimation and the different Centers ($p<0.01$); the Obesity Center had the highest percentage of patients who overestimated their CVR (33.3%).We also found that patients in the very-high-risk class had no awareness of their risk condition. This is confirmed by the fact that among patients at very high risk (27.3%) only 1.5% perceived this condition, with significant differences among the different clinics ($p<0.01$): 0 vs 31 in Hypertension-dyslipidemia Center; 3 vs 25 in Diabetes Center; 1 vs 6 in Obesity Center.
**Conclusion.** Our data showed underestimation of CVR among patients referring to our clinics expecially between very-high-risk patients underlining the need for maximize patients' risk consciousness in order to create more adherence on therapeutic strategies and lifestyle.

## SEVERE AND TRANSIENT DISAPPEARING HIGH DENSITY LIPOPROTEIN SYNDROME PUTATIVELY TRIGGERED BY AN ACUTE STRESSFUL PSYCHOLOGIC EVENT

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I report herein a case of a middle-aged very healthy woman with a history of moderate polygenic hypercholesterolemia, high levels of HDL-cholesterol (mean 80 mg/dl) and absence of clinical atherosclerotic disease or subclinical carotid atherosclerosis who, about two weeks after an acute and severe psychologic stress, developed mild general malaise and a rapidly progressive reduction of HDL-cholesterol (nadir level: 7 mg/dl) associated with blood changes suggestive of a lymphoproliferative disorder (high monocytes count with increased non-clasic CD4- cells, high lactic dehydrogenase levels, etc.). Laboratory and imaging diagnostic studies eventually excluded haematological or autoimmune diseases and, interestingly, HDL-cholesterol levels as well as all the other laboratory abnormalities spontaneously resolved within three months. While sepsis, burns, severe hepatic failure, some drugs and hematological disorders are recognized causes of Disappearing High Density Lipoprotein Syndrome (1-4), this is the first one reported, to the best of my knowledge, with psychologic acute stress as the putative trigger.

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## META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS EVALUATING THE EFFECT OF LIPID-LOWERING THERAPIES ON LIPOPROTEIN(A) LEVELS

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Large epidemiological studies, Mendelian randomized studies, and genome-wide association studies inform that elevated lipoprotein(a) [Lp(a)] concentration is an independent and potentially causal risk factor for cardiovascular diseases. However, no approved therapies for patients with elevated Lp(a) levels are available. We aimed to investigate to what extent available lipid-lowering therapies (LLTs) affect Lp(a) level. This meta-analysis was conducted according to the PRISMA guidelines. PubMed, Web of Science, EMBASE, Cochrane Library, and ClinicalTrial.gov were searched from inception to February 2023. Inclusion criteria were:
1) randomized controlled trials (RCTs) in adults (≥18 years), phase II, III or IV;
2) English language;
3) comparing the effect of LLTs vs placebo;
4) reporting the effects on Lp(a) levels;
5) with intervention duration of more than 3 weeks.
Pooled estimates were assessed by fixed-effect or random-effects model. Between-study heterogeneity was tested and measured by Cochrane's Q test and I2 statistics. Overall, 65,174 subjects from 84 RCTs were included in our meta-analysis (31 RCTs for PCSK9 inhibitors [PCSK9i], 29 RCTs for statins, 17 RCTs for omega-3 fatty acids [omega3FAs], 9 RCTs for CETP inhibitors [CETPi], 6 RCTs for fibrates, and 4 RCTs for ezetimibe). Lp(a) levels were considerably reduced by PSCK9i (-6.56 mg/dL, 95%CI -7.98 to -5.13) and CETPi (-6.60 mg/dL, 95%CI -9.26 to -3.95). Statins (-0.24 mg/dL, 95%CI -1.29 to 0.80), ezetimibe (-2.38 mg/dL, 95%CI -6.48 to 1.72), omega3FAs (-0.38 mg/dL, 95%CI -0.86 to 0.11), and fibrates (-0.70 mg/dL, 95%CI -1.51 to 0.11) did not significantly affect Lp(a) concentration.In summary, among the LLTs evaluated in our meta-analysis, PCSK9i and CETPi lower Lp(a) levels while other drugs are only minimally effective. Further research is necessary to understand whether the Lp(a) reduction translates into a clinically relevant cardiovascular benefit.

## THE RISE OF LIPOPROTEIN A IN PATIENTS ON STATIN THERAPY

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**Introdution.** Lp(a) is a lipoprotein that has a structure similar to LDL, but additionally has apolipoprotein a. Lp(a) is an independent cardiovascular risk factor and also a major determinant of residual cardiovascular risk in patients reaching the target LDL-C value. Very often people with a high plasma level of Lp(a) also have a high plasma level of LDL-C and for this reason are on statin therapy. Various scientific works have been carried out to understand the effect of statins on the plasma level of Lp(a), but the results are conflicting. Our study was created to understand whether statin therapy is associated with a change in Lp(a) over time.
**Materials and Methods.** Included in the study were patients on statin therapy, who had at least three Lp(a) measurements 6 months apart, followed by the Outpatient Clinic of the Atherosclerosis and Dyslipidemia Center of the Sant'Orsola Polyclinic from 1 January 2018 to 30 April 2023. Some of the patients had been on chronic therapy for at least 6 months, while others were naïve patients.
**Results.** 171 patients were followed, 98 of whom were on chronic therapy and 73 were naïve. Of the patients considered in the study, 135 are on low-intensity statin therapy, 36 on high-intensity statin therapy. From the analysis of the data it emerges that the average Lp(a) value in patients on chronic therapy after 6,12 and 18 months from the start of therapy is respectively (in mg/dL) 69.9-74.4-79, 4 (P=0.02). The mean Lp(a) value of naïve patients before starting therapy and after 6 and 12 months from the start of therapy is respectively (in mg/dL) 68.2-69.3-73.9 (P=0.017).
**Conclusions.** Statin therapy causes a progressive increase in Lp(a) over time. The increase in Lp(a) could reduce the effect of statin therapy in terms of reducing cardiovascular risk, but does not justify the suspension of treatment. (P=0.017).

# INDICE DEGLI AUTORI