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Effect of the deletion of Prenylcysteine oxidase 1 (PCYOX1) on arterial thrombosis in an animal model

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Prenilcistein oxidase 1 (PCYOX1) enzyme, involved in the degradation of prenylated proteins, is expressed in different types of cells, among which vascular and blood cells. Previous studies demonstrated that the secretome of cells silenced for PCYOX1 reduced platelet adhesion on both fibrinogen and endothelial cells, suggesting its possible involvement in thrombotic mechanisms.
In this study we analyzed the role of PCYOX1 in arterial thrombosis by the use of an animal model. All the procedures have been carried on mice knock-out for PCYOX1 (Pcyox1KO) that were compared with wild-type (WT) mice. Arterial thrombosis was induced by Ferric chloride application on carotid artery, while pulmonary thromboembolism was induced by the injection of collagen-epinephrine. The phenotype and the functionality of platelets were analyzed by cytofluorimetry and functional tests. The expression of PCYOX1 on platelets was evaluated by mass spectrometry.
Thrombus formation induced by Ferric Chloride was reduced in Pcyox1KO mice, that were also protected from pulmonary thromboembolism. No differences were identified in blood cells count, vascular pro-coagulant activity and functional fibrinogen. Interestingly, Pcyox1KO mice displayed a marked reduction in the number of platelets-leukocytes aggregates, in the release of alpha granules, in the activation of receptor αIIbβ3 and in platelets aggregation induced by ADP e TRAP (analyzed on whole blood or platelets rich plasma). Mass spectrometry showed that PCYOX1 was highly expressed in WT platelets. However, the deletion of PCYOX1 did not alter platelets phosphorylation pathways, and platelets adhesion and aggregation (analyzed on washed platelets), in respect of WT mice. Of note, when platelets aggregation was performed on washed platelets isolated from WT mice in the presence of plasma derived from Pcyox1KO mice, we observed a strong impairment in comparison with the aggregation obtained on the same platelets resuspended in plasma derived from WT mice.
In conclusion, our results, showing an ipo-reactivity of platelets and a reduced arterial and pulmonary thrombosis in Pcyox1KO mice, suggest that this protein could represent a new potential target in antithrombotic therapy.

Impact of dietary choline on lipid metabolism and atherosclerosis development in apoEKO mice deficient or overexpressing apolipoprotein A-I

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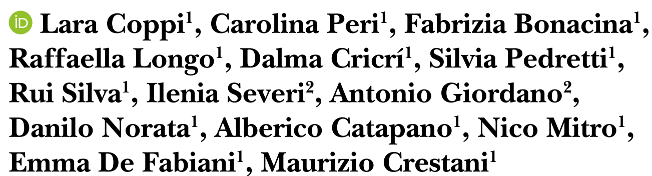
TMAO, a metabolite of dietary choline, is considered a pro-atherogenic molecule for its ability to interfere with the reverse cholesterol transport, in which apolipoprotein A-I and HDL play a key role. In the present work it was evaluated how TMAO impacts on the development of atherosclerosis in mice with different levels of apoA-I/HDL. Mice deficient in both murine apoA-I and apoE (DKO) and DKO mice overexpressing human apoA-I (DKO/hA-I), characterized by extremely low or high plasma HDL levels respectively, were fed for 16 weeks two standard rodent diets, differing only in their choline content (0.09% or 1.2%). At the end of the dietary treatment, atherosclerosis development was quantified at the aortic sinus, targeted plasma metabolomics was performed, and gene expression was evaluated in liver, duodenum, jejunum and ileum.
With both diets, DKO mice developed much larger plaques than DKO/hA-I mice. High-choline diet increased plasma TMAO levels in both genotypes. Interestingly, a worsening of plaque development by high choline diet occurred in DKO/hA-I mice only (0.057±0.048 mm^2 vs 0.0988±0.064 mm^2, $p<0.01$). Plasma metabolomics indicated that choline supplementation, only in the presence of HDL, significantly increased the concentration of some ceramide species in addition to several markers of impaired kidney function.
High-choline diet increased the hepatic gene expression of Fmo1 and Fmo2 in DKO/hA-I, whereas the expression of Scarb1 was lower in DKO/hA-I compared to DKO mice, regardless of the dietary treat-

ment. Intestinal expression of genes involved in inflammatory response and in lipid metabolism was comparable between genotypes and was not modified by choline supplementation.
In conclusion, high choline diet increased plasma TMAO concentration in both genotypes, but affected atherosclerosis development, plasma metabolome and hepatic gene expression only in high HDL mice. Intestinal gene expression was not affected neither by genotype nor by dietary choline content.

Role of histone deacetylase 3 (HDAC3) in adipose tissue metabolism and immunophenotype



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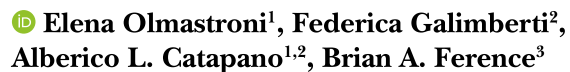
Introduction: Obesity is associated with comorbidities such as cardiovascular disease and type 2 diabetes. HDAC3 regulates adipose tissue physiology (WAT), and its genetic inactivation causes metabolic reprogramming of white adipocytes toward browning. The aim of this work is to evaluate the effect of HDAC3 silencing at different stages of differentiation and investigate the influence of adipocyte metabolism on the immunophenotype of WAT.
Materials and Methods: Following HDAC3 silencing in mesenchymal stem cells and mature adipocytes, adipocyte function, RNA, DNA and protein levels, and proliferation at the end of differentiation were analyzed. Visceral WAT immunophenotype (vWAT) of *Hdac3* KO mice in WAT (Hdac3fatKO) and controls (FL) was performed by FACS.
Results: Silencing HDAC3 in precursors amplifies the expression of genes and proteins that regulate differentiation, oxidative metabolism, browning and mitochondrial activity. Following silencing, we found increased 1) phosphorylation of AKT (1.64 fold change, $P<0.0001$), indicative of increased insulin signaling, and 2) proliferation, characteristic of the early phase of differentiation. Mitochondrial content was unchanged, but increased mitochondrial activity was observed in terms of maximal respiration (1.42 fold change, P=0.0151) and uncoupling of the electron transport chain (+11.6%, $P<0.0001$). No difference was observed following HDAC3 silencing in mature adipocytes.
We hypothesized that the enhancement of oxidative metabolism may cause cellular damage or senescence and, consequently, the immunophenotype of vWAT might be affected by HDAC3 ablation. Analysis reveals an increase of macrophages (2.48 fold change, P=0.0311) in the vWAT of Hdac3fatKO mice polarizing toward the M2 population. Coculture of adipocytes with macrophages from bone marrow indicates that HDAC3 silencing in adipocytes stimulates macrophage activation.
Conclusions: HDAC3 is a key factor in the WAT phenotype, and its inactivation triggers mechanisms that support browning. Early epigenetic events mediated by HDAC3 silencing are crucial in directing adipocyte precursors toward the oxidative phenotype. Finally, results obtained from *ex vivo* and *in vitro* studies suggest that specific factors produced by KO adipocytes may be involved in determining the observed immunophenotype.

Combining family history of coronary heart disease and individual genetic predisposition to predict the risk of major coronary events



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Background: Inherited predisposition to atherosclerosis leads to higher risk for developing coronary heart disease (CHD). There are mainly two ways to conceptualize inherited risk of CHD: family history and polygenic predisposition. We aimed at assessing the impact of family history of CHD and genetic predisposition in predicting the individual lifetime risk of major coronary events (MCE).
Methods: Using adjusted Cox proportional hazard models, we estimated the lifetime risk of MCE associated with parental family history of CHD and individual genetic predisposition (estimated by a polygenic risk score including 350 variants).
Results: A total of 445,744 UK-Biobank participants were included in the study (mean age 57 years; 54.3% females). Having one parent with a history of CHD increased the lifetime risk of MCE by 75% (HR 1.75, 95%CI 1.70-1.82). Having both parents with a history of CHD further increased the risk (HR 2.78, 95%CI 2.64-2.92) Similarly, a dose-dependent step-wise increase in MCE risk was observed moving from the lowest to the highest decile of the polygenic score. Compared to subjects without family history of CHD and with average level of the polygenic score, having a parental history of CHD determined an increase in lifetime risk of MCE (HR 1.90, 95%CI 1.82-1.98) comparable to belonging to the highest decile of the polygenic score (HR 1.89, 95%CI 1.76-2.02). However, if subjects present both parents with family history of CHD and a very high polygenic predisposition, the risk was even higher (HR 3.54, 95%CI 3.34-3.75), suggesting an additive contribution to the characterization of the lifetime risk.
Conclusions: We described the addictive impact of family history of CHD and individual polygenic predisposition in predicting lifetime risk of MCE. In order to identify subjects at higher risk of having an early event, it is essential to retrieve information about both these hereditary components.