FINAL REPORT

K115723

I. Structural and functional changes of HDL in dyslipidemic patients

Hyperlipidemia is associated with enhanced atherogenesis and with chronic low-grade inflammation in obesity. Increased generation of reactive oxygen species by polymorphonuclear leukocytes was demonstrated to play an important role in the oxidative modification of low-density lipoprotein (LDL) and chronic inflammatory processes. Myeloperoxidase (MPO) is a leukocyte-derived heme protein bound to high-density lipoprotein (HDL) and is linked to oxidative stress and enhancement of atherosclerosis. Due to its antioxidant properties, HDL-associated human paraoxonase-1 (PON1) was shown to prevent the accumulation of lipid peroxides in oxidized LDL and to inactivate bioactive oxidized phospholipids. Recent data indicate that MPO, PON1 and HDL may bind to each other, forming a ternary complex, wherein PON1 partially inhibits MPO activity and MPO inactivates PON1. Furthermore, structural and functional changes of HDL can be characterized by the measuremenet of serum levels of HDL subfractions and apolipoprotein M (ApoM).

Paraoxonase-1 and myeloperoxidase correlate with vascular biomarkers in overweight patients with newly diagnosed untreated hyperlipidemia

Increased levels of MPO and decreased PON1 activity have been reported in hyperlipidemic patients; however, their associations with other vascular biomarkers have not been completely clarified. Therefore, anthropometric parameters, serum levels of lipoproteins, inflammatory markers, MPO, PON1 levels were measured. In addition, levels of CD40 ligand (sCD40L) and asymmetric dimethyl arginine (ADMA), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were determined in 167 hyperlipidemic patients with and without vascular complications and compared their data with 32 gender and BMI matched healthy controls. We found significantly higher glucose, HbA1c, total cholesterol, LDL-C, triglyceride, Lp(a), apolipoprotein B, CRP, MPO, sCD40L, sVCAM, sICAM and oxidized LDL concentrations in patients with hyperlipidemia compared to the healthy subjects. Elevated total cholesterol, LDL-C, triglyceride, lipoprotein (a),

CRP, ADMA, sCD40L and ICAM levels, as well as higher MPO levels were found in patients with vascular complications compared to those without. Significant negative correlations were found between sCD40L, ADMA, ICAM levels and PON1 arylesterase activity, while significant positive correlations were observed between sCD40L, ADMA, ICAM and MPO levels. Our results highlight the importance of HDL-associated pro-oxidant and antioxidant enzymes in the development of endothelial dysfunction and vascular diseases in hyperlipidemia. Better understanding of these processes may improve the efficacy of cardiovascular risk prediction and anti-atherogenic treatment in hyperlipidemic patients. Our work was published in the European Journal of Vascular Medicine- VASA. (Vasa. 2017 Aug;46(5):370-376. doi: 10.1024/0301-1526/a000643)

Paraoxonase-1 arylesterase activity is an independent predictor of myeloperoxidase levels in overweight patients with or without cardiovascular complications

Myeloperoxidase (MPO), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) were shown to contribute to atherogenesis, while human paraoxonase-1 (PON1) protects against oxidative stress. Although several studies investigated these biomarkers, their associations have not been completely clarified yet. We aimed to investigate these parameters in overweight hyperlipidemic, lipid-lowering therapy-naive patients (n=167) with and without vascular complications. Patients with vascular complications (VC) had significantly higher MPO and TIMP-1 levels compared to those without vascular complications (NVC). MPO levels showed a significant negative correlation with PON1 arylesterase activity and positive correlations with MMP-9 and TIMP-1, respectively. PON1 arylesterase activity was found to be an independent predictor of MPO levels in the whole patient group or when studied separately in the subgroups with or without cardiovascular complications. Our results suggest that parallel investigation of MPO, MMP-9 and TIMP-1 levels and PON1 arylesterase activity may be a more accurate indicator of atherosclerosis, which may allow earlier treatment and therefore, improvement of treatment efficacy. (Clin Biochem. 2016 Aug;49(12):862-7)

HDL subfraction distribution and HDL function in untreated dyslipidemic patients

We investigated the structural and some functional HDL properties in newly diagnosed, untreated dyslipidemic patients and in healthy controls to evaluate the effect of dyslipidemia on the structural and functional properties of HDL characterized by the serum levels of HDL subfractions and MPO, PON1 paraoxonase and arylesterase activities and PON1 phenotyping. We hypothesized that the level and ratio of large HDL subfractions are higher, and the level and ratio small HDL subfractions are lower in dyslipidemic subjects compared to healthy controls. A lower number of small HDL subfractions may result in lower PON activities and higher MPO levels. Significantly higher glucose, hemoglobin A1c, total cholesterol, low-density lipoprotein-cholesterol, triglyceride, lipoprotein(a), apolipoprotein B, C-reactive protein, and MPO levels were found in patients compared to the healthy subjects. There were no significant differences in PON1 paraoxonase and arylesterase activities between the two study groups, but MPO/PON1 ratio was significantly higher in patients. There was a shift towards the small HDL subfractions, but only the intermediate HDL ratio was significantly lower in patients compared to controls. The results highlight the importance of HDL-associated pro- and antioxidant enzymes suggesting the possible clinical benefit of MPO/PON1 calculation and confirm that quantification of HDL-C level alone provides limited data regarding HDL's cardioprotective effect. Calculation of MPO/PON1 ratio may be a useful cardiovascular marker in dyslipidemia.

These results were published in Vessel Plus (Vessel Plus 2017 Nov 21. [Online First] https://doi.org/10.20517/2574-1209.2017.27)

Apolipoprotein M And High-Density Lipoprotein Subfraction Levels In Newly Diagnosed, Untreated Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is an autosomal dominant disorder with extremely high plasma total- (TC) and LDL-cholesterol (LDL-C) levels and increased risk of premature cardiovascular disease. Although the HDL-cholesterol (HDL-C) level is usually in the high-normal range, the structure and function of the HDL particle is impaired. The HDL-associated apolipoprotein M (ApoM) has several anti-atherogenic

properties and was found to be decreased in FH. However, its level was not studied in untreated FH patients.56 newly diagnosed, untreated patients with heterozygous FH and 32 healthy controls were enrolled. Serum lipid parameters, ApoM levels and HDL subfractions were evaluated. ApoM level was detected by ELISA. Lipoprotein subfractions were measured by gel electrophoresis (Lipoprint). FH was diagnosed using Dutch Lipid Network Criteria. Significantly higher TC, LDL-C, triglyceride and Lp(a) levels was found in FH patients compared to the controls. ApoM level was significantly higher in patients compared to controls (p<0.01). We found significant positive correlations between ApoM levels and small HDL subfraction levels both in all subjects (p<0.001) and in FH patients (p=0.05). In multiple regression analysis ApoM level was best predicted by TC level (β=0.57; p<0.001). Measurement of ApoM level in FH may contribute to understand the role of HDL structure and function enhanced atherogenesis observed in FH patients.

These results were presented as a poster presentation on 87th EAS Congress in Maastricht, The Netherlands, between the 26th and 29th of May 2019. The abstract was published in Atherosclerosis 287 (2019) e123-e288. https://doi.org/10.1016/j.atherosclerosis.2019.06.653

II. Non-lipid effects of selective LDL apheresis treatment in patients with severe heterozygous familial hypercholesterolemia

Selective LDL apheresis is an extracorporeal therapy that reduces LDL cholesterol levels in patients with severe heterozygous and honozygous familial hypercholesterolemic (FH) patients. However, LDL apheresis affects several proteins including adipokines and hepatiokines involved in atherogenesis.

Changes in serum afamin and vitamin E levels after selective LDL apheresis

LDL apheresis is an extracorporeal therapy that reduces LDL cholesterol levels in patients with severely high cholesterol, e.g. heterozygous familial hypercholesterolemic (FH) patients. Beyond its lipid lowering efficacy, LDL apheresis affects several pro- and anti-inflammatory factors involved in atherosclerosis. Human

afamin is a plasma vitamin E-binding glycoprotein primarily expressed in the liver and secreted into the bloodstream where it is partially associated to apolipoprotein A1-containing HDL (sub)fractions. Results from a recent study in transgenic mice overexpressing human afamin, a possible association between afamin and components of metabolic syndrome including hyperlipidemia have been reported. Therefore, we investigated the serum levels of HDL, ApoA1, afamin, oxidized LDL, α- and γ-tocopherol and the changes in HDL subfraction distributions in heterozygous FH patients before and after their first LDL apheresis treatment. The first treatment sessions decreased serum afamin levels by an average of 9.4%. Total cholesterol, LDL-C, HDL-C and ApoA1 levels decreased by 52.6; 61.8; 10.5; and 14.1%, respectively. We found that α- and γ-tocopherol levels markedly decreased (by 34.1 and 32.9%, respectively), while α- tocopherol/cholesterol and γ-tocopherol/cholesterol ratios significantly increased (by 41.4 and 40.3%, respectively). Oxidized LDL levels significantly decreased. There was a shift toward the larger HDL subfractions. We concluded, that LDL apheresis moderately decreases the circulating levels of afamin parallel to lowering HDL-C and ApoA1 levels. Tocopherol levels decreases markedly compared to afamin levels, however, beneficial changes in vitamin E/cholesterol ratios, oxidized LDL levels and HDL subfraction distribution were detected. These additional effects of LDL apheresis may result in further cardiovascular risk reduction in FH patients.

These results were published in J Clin Apher. 2018 Oct;33(5):569-575. doi: 10.1002/jca.21636.

Impact of selective LDL apheresis on serum chemerin levels in patients with hypercholesterolemia

Selective low-density lipoprotein (LDL) apheresis is commonly used to treat patients with familial hypercholesterolemia (FH). Chemerin is an adipokine with putative roles in the regulation of lipid metabolism. In our pilot study, we measured serum chemerin levels by enzyme-linked immunosorbent assay in six severe heterozygous FH patients before and after their first LDL apheresis treatments using the technique of direct adsorption of lipoproteins (DALI). Results: The first treatment sessions decreased serum chemerin levels by an average of 27.26 %. While following one patient, 12 months of regular LDL apheresis resulted in a permanent reduction in his

serum chemerin level. Changes in the lipoprotein subfractions measured by gel electrophoresis (Lipoprint) correlated with the reduction of chemerin levels. Furthermore, we eluted and then measured chemerin bound to the DALI column. We conclude that LDL apheresis decreases the circulating level of chemerin by binding the protein to the column and thus improves lipoprotein subfraction pattern.

These results were published in Lipids in Health and Disease (2016) 15:182 DOI 10.1186/s12944-016-0353-x

III. Adipokines: potential links between obesity and atherosclerosis

Adipokines secreted by adipose tissue are key regulators of imflammation, oxidative stress, lipoprotein metabolism and atherogenesis in obesity.

We summarized details of the correlations between paraoxonase-1 and some selected adipokines, namely leptin, adiponectin and chemerin in this review:

Paraoxonase-1 and adipokines: Potential links between obesity and atherosclerosis

Oxidative stress and chronic low-grade inflammation are major characteristics of obesity-related disorders. The dominance of pro-oxidant and pro-inflammatory mechanisms triggers insulin resistance and enhances the progression of atherosclerosis. Human paraoxonase-1 is bound to high-density lipoprotein and inhibits the oxidation of lipoproteins and reduces the degree of inflammation, hence it is considered to act against atherosclerosis. In contrast, the majority of the adipokines secreted from the enlarged white adipose tissue promote the atherosclerotic process; and altered adipokine secretion is now regarded as one of the major contributors of increased cardiovascular morbidity and mortality in obesity. Adipokine imbalance leads to decreased paraoxonase-1 activity that results in enhanced atherosclerosis; therefore, altered adipokine secretion may be predictive of cardiovascular complications in obesity. As an active organ secreting biological active substances, white adipose tissue may also act as a “fine-tuner” of immune and endocrine actions attenuating or enhancing reactions triggered by pathogens, inflammation and metabolic stimuli; and obesity, as a chronic noxious state may

perturb the proper function of this fine-tuning process. We summarized details of the correlations between paraoxonase-1 and some selected adipokines, namely leptin, adiponectin and chemerin. Further investigations are of major importance to elucidate the associations between adipokines and paraoxonase-1 and to establish accurate interventions against obesity-related disorders. Chemico-Biological Interactions http://dx.doi.org/10.1016/j.cbi.2016.04.003

Serum obestatin level strongly correlates with lipoprotein subfractions in non-diabetic obese patients

In the past few decades, it has been recognized that hormone-like proteins secreted by adipose tissue (adipokines) are important for metabolic homeostasis including lipid metabolism. However, the regulatory effect of further proteins secreted by other tissues such as gastrointestinal tract has not been clarified. Obestatin is an anorexigenic gut hormone, a ghrelin-derived peptide. Although many of its effects is unclear, accumulating evidence supports positive actions on both metabolism and cardiovascular function. Human circulating obestatin levels show an inverse association with obesity and diabetes. To date, level of obestatin and its correlations to the components of metabolic syndrome and lipid subfractions in non-diabetic obese (NDO) patients have not been investigated. Therefore, anthropometric parameters, serum levels of lipoproteins and their subfractions, inflammatory and oxidative markers, MPO, PON1, obestatin levels were measured in 50 NDO patients and compared their data with 32 age- and gender-matched healthy controls.

Serum level of obestatin was significantly lower in NDO patients compared to controls. We found significant negative correlations between the level of obestatin and BMI, level of serum glucose, HbA1c and insulin. Significant positive correlation was found between obestatin level and the levels of ApoA1 and large HDL subfractions, and mean LDL size ($r=0.25$; $p<0.05$). Serum VLDL ratio and level negatively correlated with obestatin. In multiple regression analysis obestatin was predicted only by VLDL level. Based on our data, measurement of obestatin level in obesity may contribute to understand the interplay between gastrointestinal hormone secretion and metabolic alterations in obesity.

Our work was published in the Lipids Health Dis. 2018 Mar 5;17(1):39. doi: 10.1186/s12944-018-0691-y

Circulating Afamin Level Correlates With Lipoprotein Subpopulations In Non-Diabetic Obese Patients

Afamin is partially associated with ApoA1-containing HDL subfractions in the bloodstream. Recently, we observed that LDL apheresis moderately decreases the circulating levels of afamin parallel to lowering HDL-C and ApoA1 levels. However, association of afamin with lipid subfractions is still unclear.

We enrolled fifty obese and thirty-two gender and age-matched lean subjects without manifest carbohydrate and lipid disturbances. Serum level of afamin was measured by ELISA, while HDL and LDL subfractions were determined with Lipoprint gel electrophoresis.

Circulating afamin concentration was significantly higher in the obese group compared to controls ($p<0.001$). Total HDL-C and LDL-C level did not show association with afamin level. Percentage of large HDL subfraction correlated negatively ($p<0.001$), while percentage of small HDL subfraction correlated positively ($p<0.001$) with serum afamin level in overall subjects. There was positive correlation between serum afamin level and percentage of large and small-dense LDL subfraction ($p<0.01$ and $p<0.01$, respectively); while mean LDL size correlated negatively with afamin ($p<0.001$).

Strong correlations with lipid subfractions may highlight the key role of human afamin in metabolic syndrome and obesity.

These results were presented as a poster presentation on 87th EAS Congress in Maastricht, The Netherlands, between the 26th and 29th of May 2019. The abstract was published in Atherosclerosis 287 (2019) e123-e288.

DOI: https://doi.org/10.1016/j.atherosclerosis.2019.06.498

Beside the low grade inflammation and dyslipidemia, the procoagulant milieu of obesity also contribute to increased cardiovascular risk. Previously, the relationship between plasma plasminogen activator inhibitor-1 (PAI-1) levels and the distributions of HDL

and LDL subfractions as well as the potential associations between the concentrations of PAI-1 and the HDL-linked enzymes PON1 and MPO in obese and lean non-diabetic patients have not been studied.

Plasminogen activator inhibitor-1 level and its correlation with lipoprotein subfractions in obese and lean subjects

Plasminogen activator inhibitor-1 (PAI-1) is the primary regulator of fibrinolysis and also controls cell adhesion and migration in the extravascular space, therefore it may influence the development of atherosclerotic plaque formation. High levels of PAI-1 correlates positively with thrombotic vascular conditions such as acute myocardial infarction. Therefore, elevated PAI-1 concentration is an established risk factor for coronary artery disease. In epidemiological studies, direct associations were observed between PAI-1 and lipid parameters (eg. triglyceride and HDL-C levels), however, to date, there are limited data on the association of PAI-1 with the functional and structural properties of different lipoprotein fractions. Therefore, our aim was to examine the relationship between plasma PAI-1 level and the distribution of HDL and LDL subfractions as well as to confirm potential associations between the concentrations of PAI-1 and the HDL-linked enzymes PON1 and MPO in obese and lean non-diabetic patients.

We enrolled fifty non-diabetic obese patients and thirty-two healthy volunteers. Compared to the lean subjects, levels of TNF-α, lL-6, oxLDL and MPO were found to be significantly higher, while PON1 paraoxonase and arylesterase activities were tendentiously lower in the obese patients. We detected increased concentrations of the large and small-dense LDL subfractions, while mean LDL size was significantly decreased in the obese subjects. The distribution of HDL particles showed a shift towards the small-sized HDL. Strong significant negative correlations were found between plasma PAI-1 concentration and mean LDL-size; as well as between PAI-1 concentrations and the levels of the large and intermediate HDL subfractions. Significant positive correlation was found between PAI-1 level and the concentration of small HDL subfraction. In multiple regression analysis, PAI-1 was predicted by waist circumference and intermediate HDL subfractions. Our data indicate that PAI-1 might be linked to lipid metabolism in obese patients, elevated PAI-1 level might be contributed to increased risk of atherogenesis and cardiovascular risk.

Our work was published in Int J Endocrinol. 2018 May 30;2018:9596054. doi: 10.1155/2018/9596054.

IV. Investigation of the effect of alpha-lipoic acid treatment biomarkers of endothelial function in diabetic neuropathy

Type 2 diabetes is associated with oxidative stress and enhanced atherogenesis. However, the relationship between endothelial dysfunction in type 2 diabetic patients with peripheral neuropathy after treatment of alpha-lipoic acid ***(***ALA) has not been clarifed.

Effect of alpha-lipoic acid treatment biomarkers of endothelial function in diabetic neuropathy

Diabetic neuropathy develops on a background of hyperglycemia and is associated with increased oxidative stress. Elevated asymmetric dimethylarginine (ADMA) levels are linked to oxidative stress reducing the synthesis of nitric oxide (NO) by uncoupling NO synthase. Oxidative stress induces changes in nerve conduction velocity in diabetic patients. There is strong evidence that alpha-lipoic acid (ALA) as an antioxidant may improve nerve conduction and relieve neuropathic pain. Therefore, we aimed to investigate the relationship between endothelial dysfunction and NO synthesis in type 2 diabetic patients with peripheral neuropathy after treatment of ALA. Fifty-four type 2 diabetic patients with neuropathy were included in the study. Serum ADMA concentration, ICAM-1, VCAM-1, oxLDL and TNF-alpha levels were determined with ELISA. NO concentrations were measured by the Griess reaction. Peripheral sensory nerve function was assessed by current perception threshold (CPT) testing. Autonomic function was assessed by Ewing's five standard cardiovascular reflex tests (CAS). We found that ADMA levels were significantly decreased $p<0.001$), NO levels were significantly increased ($p<0.05$) after six month of 600 mg/day ALA treatment. The CPT and CAS significantly improved after ALA treatment. The improvement of current perception threshold values was correlated positively with the change of ADMA levels ($r=0.58$, $p<0.001$). Change in ADMA level was more pronounced in responder patients based on both CPT and CAS. Our results suggest that ALA supplementation improves endothelial function in patients

with diabetic neuropathy. Changes in serum ADMA levels may predict the clinical response to ALA treatment.

These results are published in Archives of Medical Science. - "Accepted by Publisher" (2020).