SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Investigation of lipoprotein subfractions in lipid metabolic disorders

by Hajnalka Lőrincz

Supervisor: Ildikó Seres, PhD

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF HEALTH SCIENCES

DEBRECEN, 2015

Investigation of lipoprotein subfractions in lipid metabolic disorders

By Hajnalka Lőrincz, biologist MSc

Supervisor: Ildikó Seres, PhD

Doctoral School of Health Sciences, University of Debrecen

Head of the **Examination Committee**: Róza Ádány, MD, PhD, DSc

Members of the Examination Committee:
Anikó Somogyi, MD, PhD, DSc
Dániel Törőcsik, MD, PhD

The Examination takes place at the Conference Room in the Bldg. of the Faculty of Public Health, University of Debrecen 11:00, October 6, 2015

Head of the **Defense Committee**: Róza Ádány, MD, PhD, DSc

Reviewers: László Márk, MD, PhD
László Oláh, MD, PhD

Members of the Defense Committee:
Anikó Somogyi, MD, PhD, DSc
Dániel Törőcsik, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 13:00, October 6, 2015

INTRODUCTION

Lipoproteins are large molecular complexes that play a major role in transporting triglyceride, cholesterol, cholesteryl esters and other lipophilic molecules in the plasma. Circulating lipoproteins range in size from 5 to >1000 nm and containing a coat that is composed of a monolayer of amphipathic phospholipids and peripheral and integrant proteins surrounding a lipid-rich hydrophobic core. The main structural proteins of these molecular complexes are the apolipoproteins which serve not only to stabilize the particles, but also to mediate metabolic functions by acting as receptor ligands or by activating enzymatic activities that promote the metabolism of lipoproteins within the plasma compartment. Currently, the clinical evaluation of the lipoproteins focuses almost exclusively on their total cholesterol content. However, plasma lipoproteins cannot be solely characterized by their cholesterol content, since these lipoprotein particles undergo a continuous structural remodeling through various interactions with other lipoproteins, enzymes and cell surface receptors. Therefore, many observations have suggested that assessment of the lipoprotein functionality might be more informative than simple measurements of plasma lipoprotein cholesterol levels. Lipoprotein particles represent heterogeneous populations with respect to their size, density, electrophoretic mobility and lipid composition. There are several analytical methods to detect lipoprotein subfractions. Gradient ultracentrifugation and gel-electrophoresis are the most commonly employed techniques; however, nuclear magnetic resonance and the ionic mobility methods are also increasingly applied for separation of lipoprotein subfractions. The nomenclature of different subspecies varies depending on the separation technique used.

Low-density lipoprotein (LDL) was defined as a group of lipoprotein particles within the density limits of 1.019-1.063 g/ml, consisting of about 170 triglyceride and 160 cholesteryl ester molecules; and its proteome structure consists of a single copy of apolipoprotein B (apoB). The predominance of the so-called small-dense

LDL subspecies results in an increased susceptibility to oxidative modifications and subsequently enhanced atherosclerosis and increased cardiovascular risk. In turn, larger and less dense LDL subfractions considered to be less harmful in the progression of atherosclerosis.

The inverse relationship between plasma levels of high-density lipoprotein (HDL) and cardiovascular diseases has been widely accepted. HDL was traditionally defined as the d=1.063-1.21 g/ml density lipoprotein fraction. Phospholipids form the bulk of the HDL lipidome, whereas cholesteryl esters account for the 14–18%, triglycerides for the 3-6%, and free cholesterol for the 3-5% of the lipidome. Near 200 proteins have been identified in the surface of the HDL. Of these proteins, apoAI and apoAII have been identified as the most significant structural apolipoproteins. Similar to LDLs, HDLs are a class of structurally and functionally heterogeneous particles that vary in size and density. In addition, various HDL particles are not equally protective against atherosclerosis, as the large HDL subfractions are primarily ascribed to reverse cholesterol transport, while the small-sized HDL subfractions rather exert antioxidant effects through their associated anti-inflammatory enzymes.

Human paraoxonase-1 (PON1) is an HDL-associated enzyme with significant antioxidant properties located in the apoAI-containing subfraction of HDL. Its gene contains two common polymorphism sites in its coding region [PON1-192 glutamine(Q)/arginine(R) and PON1-55 leucine(L)/methionine(M)] influencing the enzyme activity and its concentration. Previous studies demonstrated that PON1 is mainly localized on the small HDL subfractions. Due to its antioxidant properties, PON1 prevents LDL from oxidative modification reducing its pro-atherogenic and pro-inflammatory effects. PON1 activity was diminished in several human diseases that are characterized by enhanced oxidative stress; however, age, environmental risks, smoking and alcohol consumption may also affect PON1 activity.

Previous studies have widely examined obesity-related lipid abnormalities and distribution of lipoprotein subfractions. Elevated level of small-dense LDL

subfraction correlates strongly with increased levels of triglyceride and decreased levels of HDL-cholesterol (HDL-C), which abnormalities are typical of atherogenic lipid profile. The proportion of the HDL subfractions shifts towards the small-sized HDL in obesity; however, the protective effect of this structurally modified HDL is limited in atherosclerosis.

It also has to be mentioned, that white adipose tissue, especially in the visceral compartment, is not only considered as a mere energy depository, but as an active endocrine organ releasing a variety of biologically active substances termed adipokines. Besides modulating adipogenesis, adipokines play fundamental roles in the regulation of lipid/glucose metabolism, insulin sensitivity and inflammation *via* their autocrine, paracrine and endocrine effects.

Chemerin is a recently described adipokine expressed primarily in the white adipose tissue, liver and kidney. It is secreted as an 18 kDa inactive pro-protein, which undergoes an extracellular serine protease cleavage of the C-terminal portion generating the 16 kDa active chemerin that is present in plasma and serum. Chemerin possesses several biological functions: it regulates the innate and adaptive immune system and it also serves as a chemoattractant for immature dendritic cells, macrophages and natural killer cells. In human studies, chemerin was found to be associated with the development of obesity and insulin resistance. Circulating chemerin levels increase with the degree of obesity and serum chemerin concentration was significantly elevated in obese subjects compared to normal-weight controls. Recent studies demonstrated tight associations between increased chemerin levels and increased triglyceride and LDL-cholesterol (LDL-C) and decreased HDL-C levels, respectively. However, there is no data about the potential correlations between chemerin levels and various lipoprotein subfractions; however, these data may provide important additional information about obesity-related qualitative changes in lipid metabolism.

Lipoprotein subfractions are involved not only in the development of obesity but also in hereditary cholesterol metabolism disorders and related syndromes.

Smith-Lemli-Opitz syndrome (SLOS) is an extremely rare autosomal recessive genetic disease which was firstly described in 1964. The genetic defect manifests as a large and variable spectrum of phenotypes, including multiple congenital malformations, learning and feeding problems, photosensitivity, auto-aggression, cataract, microcephaly and syndactyly of the second and third toes. The cause of this syndrome originates from biallelic mutations of the 7-dehydrocholesterol reductase *(DHCR7)* gene located on chromosome 11q13. Absence or defective function of the enzyme curbs the transformation of 7-dehydrocholesterol (7DHC) to cholesterol, which leads to perturbed cholesterol biosynthesis. Therefore, SLOS patients exhibit extremely high levels of 7DHC and reduced levels of cholesterol in the tissues, plasma and other fluids. The routine therapy has been cholesterol supplementation in SLOS; while combined cholesterol+simvastatin or cholesterol+antioxidant treatment might also be effective in SLOS patients. Regarding lipid peroxidation, 7DHC is a highly reactive lipid component that generates various cytotoxic oxidative agents (oxysterols). Additionally, inactivation of the endogenous antioxidant defense mechanisms may also contribute to increased oxidative stress. To date, data are scanty regarding the distributions of LDL and HDL subfractions in SLOS, while information is also lacking about the antioxidant properties of HDL in the disease.

AIMS

1. We aimed to examine the distribution of LDL and HDL subfractions and serum chemerin concentrations in non-diabetic obese (NDO) subjects, comparing their data to non-diabetic, non-obese healthy individuals.

2. We studied the potential correlations between serum chemerin concentrations and the various LDL and HDL lipoprotein subfractions. We also aimed to determine variables that best predict circulating chemerin levels.

3. We intended to examine the distributions of LDL and HDL subfractions in children with an inherited cholesterol metabolic disorder, Smith-Lemli-Opitz syndrome, comparing their parameters to healthy controls.

4. We also aimed to study the degree of the endogenous oxidative stress in SLOS by monitoring the cytotoxic 7-dehydrocholesterol levels and the activity of HDL-linked antioxidant paraoxonase-1 enzyme. Associations between PON1 activities and the distributions of lipoprotein subfractions were also investigated.

PATIENTS AND METHODS

Enrollment of non-diabetic obese patients and lean controls

We enrolled fifty NDO patients (age: 44.2±13.5 yrs, 43 females and 7 males, BMI: 41.96±8.63 kg/m^2) who were referred to our obesity outpatient clinic at Department of Medicine, University of Debrecen and 38 healthy volunteers matched in sex and age (age: 42.26±11.36 yrs, 33 females and 5 males, BMI: 24.05±3.21 kg/m^2. Obesity was defined as BMI≥30 kg/m^2. Participants with active liver or endocrine disease (including any types of diabetes mellitus), cardiovascular disease, renal impairment or malignancy were excluded. Further exclusion criteria were pregnancy, lactation, current smoking, alcoholism and lipid-lowering therapy. None of participants were on antihypertensive treatment with exception of ten NDO patients, who were on diuretics (indapamide) because of mild hypertension. All participants provided written informed consent. The study protocol was approved by the Ethical Committee of University of Debrecen and the study was carried out in accordance with the Declaration of Helsinki.

Enrollment of children with Smith-Lemli-Opitz syndrome and healthy controls

11 children with clinical and biochemical diagnosis of SLOS (aged between 0.1-20 years, 4 girls and 7 boys) and 10 healthy control children (age of 0.3-19 years, 5 girls and 5 boys) were enrolled in our study. None of the studied children received dietary and antioxidant supplements and were free of clinically significant infectious diseases. Malignancy, liver and renal impairment were also excluded. For phenotypic characterization of SLOS, the scoring system of Kelley and Hennekam has been used weighting embryologically separate organ systems equally. On the basis of the clinical severity scores, patients were assigned to three groups: (i) patients with mild SLOS (n=3, clinical severity score<20), (ii) typical SLOS (n=7, clinical severity scores 20–50) and (iii) severe SLOS (n=1, clinical severity score>50). After setting up the diagnosis, 6 patients received cholesterol

supplementation (Cholesterol Module, 50–250 mg/kg/day, Nutricia; no 18.012), which was complemented with simvastatin (dosage 0.2–0.4 mg/kg/day). All parents provided written informed consent. The study protocol was approved by the Ethical Committee of University of Debrecen and the study was carried out in accordance with the Helsinki Declaration.

Sample collection and laboratory measurements

Venous blood samples were taken after an overnight fasting and sera were separated immediately. Routine laboratory parameters – high-sensitivity C-reactive protein (hsCRP), glucose, fructosamine, uric acid, haemoglobin A1c, fasting insulin, total cholesterol, triglyceride, HDL-C, LDL-C, apoAI, apoB and lipoprotein(a) levels – were determined from fresh sera with standard laboratory measurements in the University of Debrecen, Institute of Laboratory Medicine. To confirm non-diabetic status in obese and lean participants, we applied a routine oral glucose tolerance test after fasting for 12 h. Homeostasis model assessment-insulin resistance (HOMA-IR) was also calculated.

Lipoprotein subfraction analysis

Lipoprotein subfractions were detected by an electrophoretic method on acrylamide gel based on their size, using the Lipoprint System (Quantimetrix Corp., Redondo Beach, CA). Serum samples were added to acrylamide gel tubes and applied Sudan Black as a lipophilic dye. After electrophoresis, lipoprotein fractions (bands) were identified by their mobility with Lipoware computer softver (Quantimetrix Corp.). During the Lipoprint LDL test, the proportion of the large LDL was defined as the sum of the percentage of LDL1 and LDL2, whereas proportion of small LDL was defined as the sum of LDL3-LDL7. According to the Lipoprint HDL test, up to 10 HDL subfractions were grouped into three major classes: large (HDL1-HDL3), intermediate (HDL4-HDL7) and small (HDL8-HDL10) HDL subfractions. Cholesterol concentrations of each lipoprotein bands

were calculated by multiplying the relative area under curve of subfractions by total cholesterol concentration of the sample.

Determination of serum chemerin concentration

Serum total chemerin concentrations were measured by a commercially available ELISA kit (USCN Life Science Inc., Wuhan, China) according to the recommendation of the manufacturer.

Measurements of 7-dehydrocholesterol level and human paraoxonase-1 activity

Concentration of serum 7DHC was performed by an UV spectrophotometric method at 282 nm. PON1 paraoxonase activity was analyzed at 405 nm on a microtiter plate, utilizing paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate, Sigma) as a substrate. Paraoxonase activity is expressed as units per liter of serum, where 1 unit equals 1 μmol of substrate hydrolyzed per minute. PON1 arylesterase activity was assayed with a phenylacetate substrate (Sigma) and the hydrolysis of phenylacetate was monitored at 270 nm. Arylesterase activity is expressed in U/l; 1 U is defined as 1 μmol phenylacetate hydrolyzed per minute.

Statistical analyses

Statistical calculations were performed by STATISTICA software (ver 8.0; StatSoft Inc., Tulsa, OK). The normality of data distribution was tested by Kolmogorov-Smirnov test. Comparisons between groups were performed by Student's paired t-test in case of normally distributed variables and by Mann-Whitney U-test in case of variables with non-normal distribution. Multiple regression analysis (backward-stepwise method) was performed to determine variables best predicted chemerin levels. Results were considered significant at the level of $p<0.05$.

RESULTS

Circulating chemerin concentration and distribution of lipoprotein subfractions in obese and normal weight non-diabetic subjects

Compared to normal weight controls, serum triglyceride, lipoprotein(a), uric acid, haemoglobin A1c and glucose levels were found to be significantly higher; while HDL-C and apoAI concentrations were significantly lower in the non-diabetic obese patients; however, their measurements still fell in the physiological range. Fasting insulin levels and HOMA-IR did not differ in the studied groups. Circulating chemerin levels were significantly elevated in the group of the NDO patients compared with normal weight controls (NDO: 590.08±90.29 ng/ml *vs.* controls: 404.99±127.07 ng/ml, $p<0.001$, respectively). We found a significantly increased hsCRP level in the obese patients, indicating the presence of a chronic low-grade inflammation ($p<0.001$).

Similarly to total LDL cholesterol measurements, there was no significant difference between the two groups in the proportion of the large LDL subfraction. In turn, compared with the controls, the proportion of the small-dense LDL subfraction ($p<0.001$), furthermore, the absolute amount of large ($p<0.001$) and small LDL subfraction ($p<0.001$) were significantly higher in the NDO patients. These patients had a smaller mean LDL size compared with the lean controls ($p<0.001$). Although HDL cholesterol levels were in the normal range in both studied groups, we detected a significant shift towards the small HDL subfractions in the NDO patients ($p<0.001$).

Correlations between serum chemerin concentrations and lipoprotein subfractions

Serum chemerin levels correlated positively with total LDL cholesterol levels in all studied participants ($r=0.34$; $p=0.0009$). There was a positive, although not significant, correlation between chemerin level and the proportion of large LDL ($r=0.20$; n.s.), while a significant positive association was found between chemerin

concentration and the proportion of small-dense LDL subfraction (r=0.39; p=0.0003). Also, a significant inverse correlation was detected between serum chemerin level and the LDL particle size (r=-0.37; p=0.0008).

A significant inverse correlation was observed between serum chemerin concentrations and total HDL-C levels (r=-0.24; p=0.02), as well as between serum chemerin level and the percentage of large HDL (r=-0.47; p=0.00001). In contrast, significant positive associations were found between serum chemerin concentrations and the proportions of intermediate and small HDL (r=0.23; p=0.04; and r=0.47; p=0.000008, respectively).

To clarify the impact of BMI on our findings, multiple regression analysis was performed with chemerin as the dependent variable. Indeed, chemerin turned out to be best predicted by hsCRP ($p<0.001$) and small HDL subfraction ($p<0.001$), while BMI was not an independent predictor of chemerin (p=0.895).

Distribution of lipoprotein subfractions in children with Smith-Lemli-Opitz syndrome and healthy controls

We used the clinical scoring of Kelley and Hennekam to characterize the phenotypes of SLOS children. The mean SLOS clinical severity score was 30.9±15.1. The level of 7DHC in SLOS patients was 205 (85-274) mg/l, while its concentrations were below the limit of detection in the healthy children (<0.15 mg/l). When investigating the lipid parameters, mean total cholesterol, LDL-C and HDL-C levels were measured to be significantly decreased in patients with SLOS ($p<0.0001$, $p<0.0001$ and $p<0.01$; respectively) compared to healthy children. A strong, significant negative correlation was detected between the clinical severity score and HDL cholesterol levels (r=-0.804, p=0.003).

Compared to controls, the proportion of large LDL subfraction was significantly lower ($p<0.001$), while the proportion of small-dense LDL subfraction was significantly higher ($p<0.01$) in SLOS patients. Furthermore, the mean LDL size was significantly reduced in patients with SLOS than in healthy children ($p<0.001$). According to the HDL subfraction analysis, we detected a shift towards

the larger and less dense HDL subfractions in children with SLOS. The proportion of the large HDL subfractions was significantly higher ($p<0.001$), while the proportions of intermediate and small HDL subfractions were significantly lower in SLOS children compared to healthy controls ($p<0.001$ and $p<0.01$, respectively).

Associations between serum PON1 arylesterase activity and lipoprotein parameters in children with SLOS and healthy controls

PON1 paraoxonase activity was lower in the SLOS group compared to controls; although the difference was not significant between the two groups. In turn, serum PON1 arylesterase activity was found to be significantly decreased in SLOS children compared to controls (SLOS: 64.30±52.69 U/l *vs.* controls: 107.93±26.83 U/l; $p=0.022$).

Examining the correlations between serum PON1 arylesterase activity and lipoprotein parameters, we detected a significant positive correlation between PON1 arylesterase activity and total cholesterol level ($r=0.543$, $p=0.045$). In turn, a significant negative association was found between PON1 arylesterase activity and the proportion of small-dense LDL subfraction ($r=-0.578$, $p=0.031$). Additionally, PON1 arylesterase activity correlated positively with mean LDL size ($r=0.610$, $p=0.021$). Although there was no significant association between PON1 arylesterase activity and HDL-C levels; PON1 arylesterase activity correlated negatively with the proportion of large HDL subfraction ($r=-0.798$, $p=0.001$). In contrast, significant positive correlations were found between PON1 arylesterase activities and the proportions of intermediate and small HDL subfractions ($r=0.652$, $p=0.012$ and $r=0.663$, $p=0.010$), respectively.

DISCUSSION

Previous studies demonstrated that LDL and HDL lipoproteins play an important role in the atherosclerotic process and enhanced endogenous oxidative stress. In pathological conditions, the relative and absolute amounts of LDL and HDL subfractions may be shifted leading to unfavorable changes in the lipidome and proteome structure of these lipoproteins, ultimately influencing their normal functions.

In our recent study, we investigated the distribution of lipoprotein subfractions in obese patients that are free of manifest abnormalities of carbohydrate metabolism. Although the LDL-C levels fell into the normal range even in the obese patients, we detected a significantly higher proportion and an increased absolute amount of small-dense LDL subfractions in them. The small-dense LDL particles correlate tightly with the risk of cardiovascular diseases; therefore we hypothesized that NDO subjects might possess increased cardiovascular risk. While the small-dense LDL subfraction is clearly associated with the increased risk of cardiovascular diseases, data about the atherogenic properties of the various HDL subfractions are contradictory due to their functional and structural heterogeneity. Compared to the healthy participants, we demonstrated a pronounced shift towards the smaller and denser HDL subfractions in the NDO patients. Considering our previous data, we presumably detected a more atherogenic HDL subfraction distribution in the NDO patients, despite physiological total HDL-C levels.

Elevated chemerin levels were previously described in several obesity-related conditions that represent increased cardiovascular risk. Furthermore, circulating chemerin concentration correlated positively with various markers of obesity and inflammation. Corroborating previous observations, we found chemerin levels to be elevated by about 30% in NDO patients; which, in line with the increased

hsCRP concentrations, indicates the presence of the chronic low-grade inflammation in these individuals.

Several recent studies reported that circulating chemerin concentrations associated positively with total LDL-C levels and negatively with total HDL-C levels, which findings are in line with our results. Additionally and for the first time in the literature, we investigated the relationship between lipoprotein subfractions and serum chemerin levels in non-diabetic obese patients. We demonstrated a strong and significant positive association between serum chemerin level and the most atherogenic, small-dense LDL subfraction. Furthermore, we detected a significant inverse correlation between chemerin concentration and mean LDL size. Previous studies showed that chemerin might accumulate in the atherosclerotic lesions of the aorta; while a significant positive correlation was found between aortic atherosclerosis and chemerin expression of aortic vascular smooth muscle cells and foam cells. These data support the suggested role of chemerin in atherosclerosis via its paracrine action. Therefore, chemerin might modify the composition of LDL in the atherosclerotic lesion leading to the local development of the small-sized, more atherogenic LDL particles and promoting atherosclerosis.

Contrary to the LDL subfractions, correlations between chemerin levels and HDL subfractions were not uniform. We detected a significant inverse association between chemerin concentrations and the proportion of the large HDL subfraction, while significant positive correlations were found between chemerin levels and the proportion of intermediate and small HDL subfractions. Based on our multivariate analysis, hsCRP and small HDL subfractions are the best independent predictors of chemerin. Several studies described the modification of the lipid composition of HDL in pathological conditions, but only a few examined the impact of inflammation on HDL particle size and concentration. We hypothesize that, due to the obesity-related pro-inflammatory milieu, compositional alterations might develop within the HDL particle, especially in its core lipid moiety. Such

alterations, including reduction in the free cholesterol and cholesteryl ester content, may reduce HDL size and density. Indeed, circulating chemerin correlated in our study with the small HDL subfraction which has a mitigated antioxidant effect.

We also examined the distribution of lipoprotein subfractions in SLOS children who possess inherited defect of cholesterol biosynthesis and exhibit markedly reduced serum total cholesterol level. Previous studies thoroughly investigated lipoprotein parameters and impaired cholesterol biosynthesis in SLOS; however, the distributions of LDL and HDL subfractions have not been analyzed yet. Corroborating previous data, significantly decreased total cholesterol, LDL-C and HDL-C levels were measured in our patients and we found a significant negative correlation between HDL-C level and the clinical severity score, emphasizing the putative role of HDL in the pathomechanism of SLOS.

Compared to healthy infants, we found an unexpectedly higher proportion of the small-dense LDL subfraction and a lower mean LDL size in children with SLOS. Since the predominantly smaller and denser LDL subfractions are extremely susceptible to undergo oxidative modification, their dominance contribute to the oxidative stress in SLOS. According to the HDL subfraction analyses, we observed an altered distribution of HDL subfractions in SLOS patients who were characterized by a significantly higher proportion of large-sized HDL subfraction and lower proportions of intermediate and small-sized HDL subfractions. Based upon these results, one may hypothesize that altered distribution of HDL subfractions reflects an enhanced reverse cholesterol transport compensating for the extremely low cholesterol levels found routinely in SLOS; however, further studies are needed to clarify such association in this disease.

PON1 is widely recognized as one of the major antioxidant enzymes of HDL and localized primarily on small-sized HDL subfractions. Diminished PON1 activity was reported in several disease entities involving altered redox balance and increased oxidative stress. Therefore, lower proportion of small-sized HDL subfractions and decreased PON1 arylesterase activity in SLOS patients might be

related to their impaired synthesis in the liver and contributing to the reduced antioxidant capacity. It must be noted, that PON1 possesses various enzyme activities with divergent substrate specificities including paraoxonase and arylesterase activities. PON1 paraoxonase activity is determined by the PON1-192QR polymorphism in the coding region and shows a large inter-individual variation among the general population. In contrast, PON1 arylesterase activity is not affected by this polymorphism and reflects the actual protein concentration and antioxidant capacity of PON1. Compared to controls, we found a marked but not significant reduction of serum PON1 paraoxonase activity in children with SLOS. Presumably due to the relatively small sample size and the trimodal distribution of the PON1 paraoxonase activity, we did not detect a difference in this variable between the studied groups. In contrast, PON1 arylesterase activity was significantly altered in the SLOS group and showed several associations with lipoprotein subfractions. These correlations support previous observations that small-sized HDL subfraction may have the highest PON1 activity. Thus, one might conclude that decreased PON1 arylesterase activity and altered lipoprotein subfractions may serve as novel biomarkers of the adverse oxidative milieu in SLOS.

In line with decreased PON1 arylesterase activity, we also found markedly increased levels of 7DHC that further enhance oxidative stress in SLOS. 7DHC is highly disposed to react with molecular oxygen producing over a dozen different oxysterols. Since 7DHC and 7DHC-derived oxysterols are potent pro-oxidant agents, these molecules may also contribute to enhanced lipid peroxidation and protein degradation resulting in reduced PON1 activity antioxidant capacity.

SUMMARY

Plasma lipoproteins are heterogeneous particles that consist multiple lipoprotein subpopulations and vary in structure, function, size and density; therefore, their distribution might change in several lipid metabolic disorders. Indeed, lipoprotein subfractions are also associated with inflammatory and oxidative markers. Chemerin is a recently described adipokine expressed primarily in the white adipose tissue and showing positive correlations with obesity- and inflammatory-related risk factors. Human paraoxonase-1 (PON1) is an HDL-linked antioxidant enzyme with diminished activity in diseases characterized by enhanced oxidative stress.

First, we aimed to study the relationship between the distribution of lipoprotein subfractions and serum chemerin levels in non-diabetic obese (NDO) subjects. Furthermore, we also examined the associations between the distribution of lipoprotein subfractions and PON1 activity in children with an inherited cholesterol metabolic disorder termed Smith-Lemli-Opitz syndrome (SLOS).

Serum chemerin concentration was significantly higher in NDO patients and correlated positively with the highly atherogenic small-dense LDL subfraction. Compared to the lean subjects, we demonstrated a shift towards the small-sized HDL subfractions in the NDO patients. Also, a significant negative correlation was found between serum chemerin concentration and the proportion of large HDL subfraction, while there were significant positive correlations between chemerin levels and proportions of intermediate and small HDL subfraction. Based upon the multivariate analysis, hsCRP and small HDL subfraction turned out to be the best independent predictors of chemerin.

Analyzing data from children with SLOS, the levels of the cytotoxic 7-dehydrocholesterol were significantly higher in them, compared to healthy infants. We also observed a shift towards the small-dense LDL and the large HDL subfractions in SLOS. PON1 arylesterase activity was significantly decreased in

the affected children and correlated negatively with the proportions of the small-dense LDL and the large HDL subfractions. Significant positive associations were detected between PON1 arylesterase activity and the proportions of intermediate and small HDL subfractions.

In conclusion, chemerin may be involved in the regulation of lipoprotein metabolism in non-diabetic obese subjects. Early changes in the distribution of the lipoprotein subfractions may contribute to the progression of atherosclerosis, leading to increased cardiovascular risk. Decreased PON1 activity and deleterious shifts in the distribution of lipoprotein subfractions may also contribute to the impaired antioxidant status observed in SLOS; therefore, monitoring of serum PON1 arylesterase activity may serve as a complementary biomarker in SLOS.

The work is supported by a grant from the Hungarian Scientific Research Fund (OTKA 84196), by the TÁMOP-4.2.1./B-09/KONV/2010-0007, TÁMOP-4.2.2/B-10/1-2010-0024 and TÁMOP-4.2.2.A-11/1/KONV-2012-0031 projects. The TÁMOP projects are co-financed by the European Union and the European Social Fund.

Registry number: DEENK/43/2015.PL
Subject: Ph.D. List of Publications

Candidate: Hajnalka Lőrincz
Neptun ID: G3XUPQ
Doctoral School: Doctoral School of Health Sciences
MTMT ID: 10037740

List of publications related to the dissertation

1. **Lőrincz, H.**, Harangi, M., Oláh, A.V., Szabó, G.P., Fülöp, P., Somodi, S., Paragh, G., Seres, I.: Altered lipid subfraction profile and impaired antioxidant defense of high-density lipoprotein in Smith-Lemli-Opitz syndrome.
Pediatr. Res. 77, 703-709, 2015.
DOI: http://dx.doi.org/10.1038/pr.2015.33
IF:2.84 (2013)

2. **Lőrincz, H.**, Katkó, M., Harangi, M., Somodi, S., Gaál, K., Fülöp, P., Paragh, G., Seres, I.: Strong correlations between circulating chemerin levels and lipoprotein subfractions in nondiabetic obese and nonobese subjects.
Clin. Endocrinol. 81 (3), 370-377, 2014.
IF:3.353 (2013)

List of other publications

3. Fülöp, P., Seres, I., **Lőrincz, H.**, Harangi, M., Somodi, S., Paragh, G.: Association of chemerin with oxidative stress, inflammation and classical adipokines in non-diabetic obese patients.
J. Cell. Mol. Med. 18 (7), 1313-1320, 2014.
DOI: http://dx.doi.org/10.1111/jcmm.12282
IF:3.698 (2013)

4. Gaál, K., **Lőrincz, H.**, Seres, I., Harangi, M., Oláh, A.V., Paragh, G.: Characterization of a novel high-density lipoprotein antioxidant capacity assay and its application to high-density lipoprotein fractions.
Clin. Biochem. 46 (9), 825-827, 2013.
IF:2.229

Total IF of journals (all publications): 12,12
Total IF of journals (publications related to the dissertation): 6,193

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

06 May, 2015