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

The effect of overweight and obesity on the level of lipid components, oxidative and vascular markers influencing formation of atherosclerosis

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The Examination takes place at the Conference room of Department of Preventive Medicine, Faculty of Public Health, University of Debrecen, on November 15, 2018, at 11 a.m.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, on November 15, 2018, at 1 p.m.

INTRODUCTION

Today the prevalence of obesity is increasing worldwide, leading to numerous complications and co-morbidities. In the majority of cases, there is a disturbance between the balance of energy input and output, resulting in significantly increased body fat mass. Generally speaking, the higher body mass index (BMI) is associated with greater health risk compared to normal weight individuals. It increases the risk of cardiovascular diseases, hypertension, type 2 diabetes mellitus, lipid abnormalities and cancers. As a result, a higher BMI means a higher mortality rate. In our studies, we focused on the role of cardiovascular risk associated to obesity and overweight, including increased oxidative stress, impaired vascular function, and modified gastrointestinal hormonal regulation.

The relationship between obesity, inflammation, oxidative stress and atherosclerosis has been in the center of attention for many years. The development of atherosclerosis is a complex process in which oxidative processes play an important role. Various inflammatory processes induce neutrophil cell migration to the vascular wall and production of the myeloperoxidase (MPO) enzyme, which is involved in the host defense against bacteria via the production of reactive oxygen radicals. However, it also promotes oxidative low-density lipoprotein (LDL) and high-density lipoprotein (HDL) formation, thus reduces the amount of functional HDL particles. The oxidation of LDL particles enhances LDL recruitment by the scavenger receptor (SR) of the vascular macrophages, thus promotes foam cell formation. MPO damages the activity of HDL-bound antioxidant human paraoxonase-1 (PON1) enzyme by chlorination and carbamylation of the protein. In many clinical studies, MPO activity and protein synthesis proved to be an independent risk factor for atherosclerosis. By contrast, PON1 bound to the HDL surface and protects it against LDL oxidation, thus inhibits the atherogenesis, therefore, PON1 is atheroprotective. Decreased PON1 activity has been observed in a number of disorders in which the risk of atherosclerosis is increased.

To date, these vascular biomarkers have been studied mainly in patients on statin or statin-ezetimibe treatment; however, it is well known that lipid lowering agents are able to modify their serum levels significantly. Thus, the aim of the present study was to determine the changes in CD40 ligand (sCD40L), asymmetric dimethyl arginine (ADMA), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) levels, in MPO concentrations as well as in PON1 activity; and

to evaluate their relationship with vascular complications and their correlation in untreated hyperlipidemic patients.

The gastrointestinal tract produces a number of hormone-like proteins that regulate the motility of the gastrointestinal tract, thereby affecting the appetite, the function of the liver and pancreas. Moreover, they also affect several inflammatory and oxidative processes. Obestatin is an energy balance regulator peptide hormone associated to ghrelin, participating in the energy metabolism by reducing dietary intake and body weight. Furthermore, it has a beneficial effect on the function of the cardiovascular system. We investigated the possible associations between the concentration of obestatin and the function of the HDL particle characterized by HDL-linked anti- and proatherogenic enzymes: PON1 and MPO.

OBJECTIVES

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

1. Our aim was to examine serum lipid levels.
2. To determine paraoxonase and arylesterase activity of antioxidant HDL-associated PON1 enzyme, and to determine the level of prooxidant MPO.
3. To measure serum levels of vascular biomarkers including sICAM-1, sVCAM-1, ADMA and sCD40L.
4. To investigate the correlations between PON1 activity and MPO level and serum sICAM-1, sVCAM-1, ADMA and sCD40L levels.

Serum obestatin level strongly correlates with lipoprotein subfractions in nondiabetic obese patients:

5. Our aim was to examine serum lipid levels.
6. To determine LDL and HDL subfractions.
7. To measure the level of serum obestatin.
8. We also investigated the HDL-associated anti- and pro-atherogenic enzymes: human paraoxonase-1 (PON1) and myeloperoxidase (MPO).
9. We also aimed to investigate the correlations between the level of serum obestatin and lipid fractions and subfractions.

PATIENTS AND METHODS

Enrollment of overweight, hyperlipidemic patients with and without vascular complications

The study was carried out in accordance with the Declaration of Helsinki of World Medical Association and was previously approved by local and regional ethics committees. All investigated subjects gave their written informed consent to participate in the study. We enrolled 32 healthy subjects as a control group and 167 patient with newly diagnosed Fredrickson type IIa and IIb hyperlipidaemia that were referred to our obesity outpatient clinic. Physical examination and carotid ultrasound were performed regularly. Other imaging techniques (Doppler ultrasound and computer tomography) were performed in case of complaints or abnormal physical and electrocardiography (ECG) examinations. We examined the presence of hypertension, type 2 diabetes mellitus, and smoking habits in all patients. Hypertension was defined as continuous use of antihypertensive drugs or systolic blood pressure ≥ 140 mmHg, diastolic BP ≥ 90 mmHg. Type 2 diabetes mellitus was diagnosed by recurrent use of antidiabetic drugs or insulin or a fasting blood glucose level of ≥ 7 mmol/L. Smoking status was defined as previous (in the past 10 years, lasting longer than six months) and currently smoking. Overweight was defined according to the subjects' body mass index (BMI: 25 kg/m2 – 29.99 kg/m2). Physical examination and carotid ultrasound were performed regularly. Other imaging techniques (Doppler ultrasound and computer tomography) were performed in case of complains or abnormal physical and electrocardiography (ECG) examinations. Study subjects were divided into two gender-matched subgroups as (i) patients with pre-existing vascular complications and (ii) patients without vascular complications. Vascular complications were defined as ischemic heart disease (myocardial infarction or coronary sclerosis), ischemic cerebrovascular disease (ischemic stroke, transient ischemic attack, carotid artery stenosis/occlusion), and peripheral arterial disease. Vascular complications were established by patient history or upon the results of imaging techniques. Study subjects were assigned to the "patients with vascular complications" group, if at least one complication occurred. At the time of the enrolment, patients were free of acute complains. Exclusion criteria included previous and ongoing lipid lowering therapy, autoimmune disease, chronic inflammatory conditions, and active liver or endocrine disease including type 1 diabetes mellitus, malignancy, and end-stage kidney failure.

Enrollment of non-diabetic obese (NDO) patients

We enrolled fifty non-diabetic obese patients that were referred to our obesity outpatient clinic at Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Hungary, and thirty-two healthy volunteers matched in sex and age. 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. Obesity was defined as BMI ≥ 30 kg/m2. 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, and alcoholism or drug dependence. Neither obese subjects nor lean healthy controls were taking lipid lowering, hyperglycemic, anti-inflammatory, antithrombotic medications or dietary supplements. None of participants were on antihypertensive treatment with exception of ten obese patients, who were on diuretics (indapamide) because of mild hypertension.

Sample collection and laboratory measurements

All venous blood samples were collected after 12-h of fasting. The routine laboratory parameters including fasting glucose, fructose amine, high sensitive C-reactive protein (hsCRP), total-cholesterol, triglyceride, HDL-C, LDL-C, apolipoprotein A1 (apoA1), apolipoprotein B (apoB) and lipoprotein(a) levels were determined from fresh sera with Cobas c501 analyzer (Roche Ltd., Mannheim, Germany) according to the manufacturer's instruction. To check non-diabetic status in study participants, we applied a routine 75 g oral glucose tolerance test (OGTT) after an overnight fast. At the same time, hemoglobin A1c (HbA1c) and fasting insulin were also performed according to the standard laboratory techniques. Homeostasis model assessment – insulin resistance (HOMA-IR) was calculated with the formula of Matthews et al. Sera were kept frozen at -70 °C for subsequent lipoprotein subfraction analysis and for enzyme-linked immunosorbent assay (ELISA) measurements.

Paraoxonase-1 activities

PON1 paraoxonase activity was analyzed on a microtiter plate by a kinetic, semi-automated method using paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate, Sigma Aldrich) as a substrate. Enzyme activity was calculated using the molar extinction coefficient 17600 M- 1 cm-1. The PON1 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 Aldrich) and the hydrolysis of phenylacetate was monitored at 270 nm. Enzyme activity was calculated using the molar extinction coefficient 1,310 M-1cm-1. Activity of PON1 arylesterase is expressed in U/mL; 1 U is defined as 1 μmol phenyl acetate hydrolyzed per minute.

Finally, PON1 phenotype was calculated by the dual substrate method. The genetic polymorphism at codon 192 QR is responsible for the presence of two isotypes: A (low activity) and B (high activity). The ratio of the hydrolysis of paraoxon in the presence of 1 mol/l NaCl (salt-stimulated paraoxonase) to the hydrolysis of phenyl acetate was used to assign individuals to one of the three possible PON1 phenotypes: AA (low activity), AB (intermediate activity), and BB (high activity). Cut-off values between phenotypes were as follows: ratio below 3.0 for AA, ratio between 3.0 and 7.0 for AB, and ratio over 7.0 for BB phenotype.

ADMA, sCD40L, MPO, sICAM, sVCAM, oxLDL and obestatin enzyme-linked immunosorbent assays

Serum ADMA concentrations were measured by commercially available competitive enzyme-linked immunosorbent assay (ELISA) kit (DLD Diagnostika GmbH, Hamburg, Germany). Serum concentrations of sCD40L, MPO, sVCAM-1, and sICAM-1 were measured by a commercially available sandwich ELISA kit (R&D Systems Europe Ltd., Abington, England). Serum concentrations of oxidized LDL (oxLDL) were detected by a commercially available solid phase two-site enzyme immunoassay (ELISA) kit (Mercodia AB, Sweden). Plasma human obestatin was determined by EIA kit (Yanaihara Institute Inc., Shizuoka, Japan).

Lipoprotein subfraction analyses

HDL and LDL subfractions were detected by an electrophoretic method on polyacrylamide gel with the Lipoprint System (Quantimetrix Corp., CA, USA) according to the manufacturer's instructions. Seven LDL subfractions are separated from each other during the determination of the LDL subfractions and subdivided into HDL subfractions there are ten HDL subfractions. In between, up to seven LDL subfractions were distributed.
Proportion of large LDL (large LDL %) was defined as the sum of the percentage of LDL1 and LDL2, whereas proportion of small LDL (small-dense LDL %) was defined as the sum of LDL3-LDL7. Cholesterol concentrations of LDL subfractions were determined by multiplying the relative AUC of subfractions by total cholesterol concentration of the sample. Calculated total LDL-C is comprised of the sum of the cholesterol in Midbands C through A and LDL subfractions (LDL1- LDL7); and correlates strongly with the directly measured LDL-C Ten HDL subfractions were differentiated between VLDL+LDL and albumin peaks, and were grouped into three major classes: large (from HDL1 to HDL3), intermediate (from HDL4 to HDL7) and small (HDL8 to HDL10) HDL subfractions. Cholesterol concentrations of the HDL particle subsets were calculated with Lipoware software (Quantimetrix Corp., CA, USA) by multiplying the total HDL-C concentration of the samples by the relative area under the curve (AUC) of the subfraction bands.

Statistical methods

Statistical analysis was performed by STATISTICA version 8.0 (Statsoft Inc., Tulsa, OK, USA). The normality of data distribution was tested by Kolmogorov-Smirnov test. Data were presented by descriptive analysis (means ±SD in case of normal distribution, or medians [lower quartile – upper quartile] in the case of non-normal distribution). Comparisons between groups were performed by Student's unpaired t-test in case of normally distributed variables and by Mann-Whitney U-test in case of variables with non-normal distribution. Correlations between continuous variables were assessed by linear regression analysis using Pearson's test. Since the distribution of some variables of interest became normal upon base-10 logarithm transformation, we used the log values for correlation analyses. Multiple regression analysis was performed to determine which variables best predicted obestatin concentrations. Results were considered to be significant at the level of $p < 0.05$.

RESULTS

Investigation of serum paraoxonase activity and myeloperoxidase level correlation with lipid levels and certain vascular markers in untreated hyperlipidemic patients

We found significantly higher glucose, HbA1C, total cholesterol, LDL-C, triglyceride, apolipoprotein B, CRP, MPO, sCD40L, sVCAM, sICAM, and oxLDL levels in patients compared to the healthy subjects. Significantly higher total cholesterol, LDL-C, triglyceride, lipoprotein(a), CRP, and uric acid levels were found in patients with vascular complications compared to those without any abnormalities. Serum glucose and HbA1C levels were also significantly higher in patients with vascular complications, although these parameters remained in the normal range. Mean age of patients with vascular complications was significantly higher as well compared to patients without vascular complications. Furthermore, concentrations of ADMA, sCD40L, sICAM-1, and MPO were significantly higher in patients with vascular complications. We could not find significant differences in PON1 paraoxonase and arylesterase activities or in oxLDL and sVCAM-1 levels between the two study groups. Significant negative correlations were detected between PON1 arylesterase activities and the concentrations of sCD40L ($p = 0.0262$), ADMA ($p = 0.0012$), and sICAM-1 ($p = 0.0398$), respectively, in the whole study group. We observed significant positive correlations between the concentrations of MPO and sCD40L ($p < 0.0001$), ADMA ($p < 0.0001$), and sICAM-1 levels ($p = 0.0282$), respectively, in all study participants. After separately analyzing these associations in the subgroups, all correlations remained significant only in patients without vascular complications. To test whether the associations detected in univariate analyses were independent of other laboratory parameters, we carried out multiple regression analysis with MPO levels as the dependent variable. The model included sCD40L, sICAM-1, ADMA, and HDL-C levels as well as PON1 arylesterase activity. All the other clinical and laboratory parameters including age, BMI, and waist circumference did not show any significant correlations with MPO concentrations, and were therefore not included. As our results showed, MPO concentration turned out to be best predicted by sCD40L and ADMA levels in the whole study population (sCD40L: $ß = 0.338$; $p = 0.0001$ and ADMA: $ß = 0.381$; $p = 0.0001$, respectively) and in patients without cardiovascular complications (sCD40L: $ß = 0.319$; $p < 0.0001$ and ADMA: $ß = 0.440$; $p < 0.0001$, respectively). However, in patients with vascular complications

only PON1 arylesterase activity predicted MPO levels independently and negatively (ß = -0.410; p = 0.005).

The correlation of serum obestatin levels with lipid parameters, lipid subfractions, MPO and paraoxonase-1 activity in non-diabetic (NDO) individuals

The NDO patients had extremely high BMI and slightly elevated hsCRP level compared to lean individuals. Although, there were several other differences in the laboratory parameters in NDO patients compared to lean controls, these data were remained in the physiological range. Plasma triglyceride and lipoprotein(a) concentrations were found significantly higher, while the levels of HDLC and apoAI were significantly lower in the obese group compared to normal weight controls. HbA1C level was significantly higher in the obese individuals compared to controls. Fasting glucose was in normal range in both groups and the blood glucose levels at 120 min of OGTT were not elevated in the obese group. On the basis of these laboratory parameters the obese patients involved in this study have neither diabetes nor impaired glucose tolerance. Significantly higher very low-density lipoprotein (VLDL), large LDL, small LDL and small HDL levels, while significantly lower intermediate density lipoprotein (IDL), mean LDL size, large HDL and intermediate HDL levels were found in NDO patients compared to control population. Serum level of obestatin was significantly lower in NDO patients compared to controls (3.01 ± 0.5 vs. 3.29 ± 0.6 μg/ml, $p < 0.05$). We found significant negative correlations between obestatin levels and BMI ($r = -0.33$; $p < 0.001$), serum glucose levels ($r = -0.27$, $p < 0.05$), HbA1c ($r = -0.38$; $p < 0.001$) and insulin ($r = -0.34$; $p < 0.05$). Significant positive correlation was found between obestatin level and the levels of ApoA1 ($r = 0.25$; $p < 0.05$), the ratio in % of large HDL subfractions ($r = 0.23$; $p < 0.05$) and the level of large HDL subfractions (0.24; $p < 0.05$). Small HDL subfraction ratio in % showed negative, but non-significant correlation with obestatin level (−0.21; $p = 0.06$), while small HDL level did not show any correlation with obestatin. We detected significant positive correlation between obestatin level and mean LDL size ($r = 0.25$; $p < 0.05$). Significant negative correlations were found between obestatin and ratio of VLDL in % ($r = -0.32$; $p < 0.01$) and VLDL level ($r = -0.21$; $p = 0.05$), while there were significant positive correlations between obestatin and ratio of IDL in % ($r = 0.25$; $p < 0.05$) and IDL level ($r = 0.23$; $p < 0.05$). Increased oxLDL and MPO levels were found in NDO patients compared to control population. PON1 paraoxonase and arylesterase acivities

did not differ significantly between patients and controls. We could not find any significant correlations between obestatin and the levels of MPO and PON1 paraoxonase and arylesterase activities. In multiple regression analysis obestatin was predicted only by VLDL level.

SUMMARY OF THE RESULTS

In untreated, overweight patients with Fredrickson IIa and IIb hyperlipidemia and in healthy controls:

1. Significantly higher total cholesterol, LDL-C, triglyceride, ApoB, Lp (a) and oxLDL levels were found in dyslipidemic patients compared to control populations. In patients with vascular disease, except for ApoB, the same parameters were significantly higher than those with non-vascular disease.
2. Serum paraoxonase and arylesterase activities of the HDL-associated antioxidant PON1 enzyme were not significantly different in the patient and control population. Nor when compared to a patient group with or without vascular disease.
3. The prooxidant MPO level was significantly higher in patients compared to controls, respectively.
4. Serum levels of sICAM-1, sVCAM-1 and sCD40L were significantly higher in patients compared to controls. Significantly higher levels of ADMA, sCD40L and sICAM-1 were measured in patients with vascular disease compared to patients with non-vascular disease.
5. The arylesterase activity of PON1 showed significant negative correlation with serum sICAM-1, ADMA and sCD40L levels in dyslipidemic patients.
6. MPO level showed a significant positive correlation with serum sICAM-1, ADMA and sCD40L levels. In multiple regression analysis, the sCD40L and ADMA levels were independent predictors of the MPO level for the entire patient population.

In non-diabetic obese patients and healthy controls:

7. Significantly higher levels of triglycerides and Lp (a) and significantly lower HDL-C and ApoA1 levels were observed in obese patients compared to the control population, but these values were within the normal reference range. OxLDL levels were significantly higher in obese patients compared to controls.
8. We found that VLDL, large LDL, small LDL and small HDL subfractions were significantly higher in obese patients compared to healthy controls. The average LDL size was significantly lower in patients compared to controls.
9. Significantly lower serum levels of obestatin were found in obese patients compared to control populations.

10. The antioxidant PON1 paraoxonase and arylesterase activities showed no significant differences in the two study groups.
11. The prooxidant MPO level was significantly higher in obese patients compared to controls.
12. Serum levels of obestatin showed significant negative correlation with BMI, HbA1c, serum glucose and insulin, but not correlated with PON1 activities and MPO level.
13. Serum level of obestatin was positively correlated with the ApoA1 level and the amount and ratio of the large HDL subfraction, the level and the ratio of IDL subfraction and the average LDL size. Negative correlation was found with the amount and ratio of VLDL subfraction. Based on the result of the multiple regression analysis, VLDL proved to be the only significant negative predictor for obestatin levels.

DISCUSSION

Obesity and overweight, as well as increased atherosclerosis resulting arterial complications, are becoming more and more common worldwide, so these researches are also of paramount importance to better understand the association between weight gain and atherosclerosis.

To the best of our knowledge, the significant negative associations between sCD40L, ADMA, sICAM-1 levels, and PON1 arylesterase activity, as well as the significant positive correlations between sCD40L, ADMA, sICAM-1, and MPO levels in untreated hyperlipidemic patients are novel results. Multiple regression analysis proved that sCD40L and ADMA levels were independent predictors of MPO levels in the whole study population and in patients without vascular complications, while in patients with vascular complications, only PON1 arylesterase activity was a negative predictor of MPO concentration.

Previous studies proved that both statins and ezetimibe could alter the concentrations of these biomarkers, including significant alterations in the plasma levels of lipoprotein(a) and MPO. Furthermore, circulating concentrations of CRP and sICAM-1 were found to be decreased in patients receiving both simvastatin and ezetimibe. Therefore, associations between vascular biomarkers might be different in untreated patients and enrolment of treatment-naive hyperlipidaemic patients could provide additional valuable information about their vascular effects. Polymorphonuclear leukocytes are potential sources of superoxide radicals and inflammatory mediators. Their priming contributes to chronic systemic oxidative stress and low-grade inflammation leading to endothelial dysfunction, accelerated atherosclerosis, and increased prevalence of cardiovascular complications. Indeed, a decrease in polymorphonuclear leukocyte-MPO levels with increased levels of serum MPO were found in hyperlipidemic patients. Based on these findings, the positive correlations between the concentration of MPO and sCD40L, ADMA, sICAM-1 levels may highlight the role of polymorphonuclear leukocytes in the atherosclerotic process. Additionally, ADMA was found to impair nitric oxide synthesis of polymorphonuclear leukocytes, resulting in increased leukocyte adhesion to endothelial cells, superoxide generation, and MPO release. The significant association between MPO and ADMA levels may prove the ADMA/MPO interaction concept.

A previous study has provided strong evidence that low PON1 activity predicts future risk for CVD. Still, the exact endogenous substrates and the mechanisms with which PON1 protects against atherosclerosis are still

unknown. The activity of the enzyme is usually measured with paraoxon as a substrate (paraoxonase activity) to characterize its antioxidant capacity; and with phenyl acetate (arylesterase activity) which is considered to correlate with its serum level. It has been proposed that paraoxonase proteins including PON1 primarily possess lactonase activity. Noteworthy, 5-hydroxyicosatetraenoic acid (5-HETE) lactone is metabolized by PON1, possibly resulting in a modulation of the local anti-inflammatory response. Since arylesterase activity correlates with the enzyme's lactonase activity, one may hypothesize that negative correlations between PON1 arylesterase activity and ADMA, sCD40L, and sICAM-1 levels may reflect the anti-inflammatory effect of PON1. Interestingly, PON1 arylesterase activity was found to be an independent predictor of MPO levels only in patients with vascular complications, while this association disappeared in patients without vascular complications. The favorable properties of PON1 could contribute considerably to the capacity of HDL to inhibit atherosclerosis. However, systemic and vascular inflammation have been suspected to convert HDL to a dysfunctional form with impaired antiatherogenic effects. Furthermore, an elegant study proved the existence of an HDL-MPO-PON1 ternary complex, in which each partner functions as a reciprocal modulator of the other's activity, influencing oxidant stress and lipid peroxidation during inflammation. Our previous study on the same patient population also underlines the importance of this reciprocal inhibition.

Overall, our results and literature data suggest that increased production of MPO is due to activation of neutrophilic cells in untreated hyperlipidemic patients, resulting in increased MPO activity and consequently reactive free radical production. Due to the decreased PON1 activity and increased serum MPO level antioxidant and anti-inflammatory efficacy of the HDL particle is reduced. Increased oxidative stress leading to increased cell surface expression of the adhesion molecules, including sICAM-1. In parallel, increased oxidative stress is induced in the activation of platelets and their enhanced sCD40L production. On the surface of platelets, cleavage of CD40L is performed by matrix metalloproteases is produced in large amounts during arterial inflammation. SCD40L binds to the CD40 receptor on the surface of endothelial cells, which partly reduces nitric oxide production through inhibition of nitric oxide synthase, further enhances vascular matrix metalloprotease production and cell surface expression of adhesion molecules. Increased level of sCD40L also results in the activation of further inflammatory cells. Thus, these processes reinforce the formation of vascular

inflammation, which is one of the first steps in the atherogenesis and the most important cause of impaired endothelial function.

The relationship between the function of the digestive tract and the regulation of lipid metabolism can be considered as plausible, still, the related literature data is rather incomplete. However, in the last decades number of proteins with hormone-like effects have been identified which are produced by the digestive tract. One of them is obestatin.

Obestatin acts as an anorectic hormone that decreases food intake, slowed gastrointestinal motility and therefore reduces weight gain. Previous studies in humans showed significantly lower plasma obestatin levels in diabetic or non-diabetic obese subjects compared to lean controls but failed to assess diabetes mellitus or impaired glucose tolerance status. We found similar results in our obese subjects without diabetes. The exact role of obestatin in regulation of lipoprotein levels is not completely clarified. To the best of our knowledge, correlations between obestatin levels and lipoprotein subfractions have not been investigated previously.

We found a significant positive correlation between obestatin level and the levels of ApoA1 and large HDL subfractions, which may indicate a possible connection between the abnormal gastrointestinal response and decreased hepatic ApoA1 expression in obesity. Furthermore, serum VLDL ratio and level negatively correlated with obestatin, which may be explained by the previously described association between the serum level of obestatin and carbohydrate metabolism, since insulin resistance and the higher level of serum glucose result in increased hepatic free fatty acid production leading to elevated VLDL level. Moreover, in multiple regression analysis VLDL level was the only independent predictor of obestatin level. The negative VLDL correlation may also explains the positive correlation of large HDL subfraction with obestatin levels, which was on the border of significance ($p = 0.06$) in multiple regression analysis. Increased transport of triglyceride from VLDL to HDL and cholesterol-esther from HDL to VLDL by cholesterol-esther transfer protein lead to the formation of smaller and denser HDL particles with enhanced degradation and lower half lifespan, which results in low total HDL-C levels and a shift towards smaller HDL subfractions. We also investigated the activity of human paraoxonase-1, an antioxidant enzyme mainly associated with smaller HDL particles containing apolipoprotein J (clusterin). Although both paraoxonase and arylesterase activities of the enzyme tended to be lower in obese subjects, there were no significant differences in enzyme activities

between the two study groups, despite the shift towards the smaller HDL subfractions.

Furthermore, we found no significant correlation between obestatin levels and PON1 enzyme activity. The level of another HDL associated, proatherogenic enzyme: MPO was also investigated. In line with some previous studies we found significantly higher myeloperoxidase level in obese subjects compared to lean controls. Previous data showed that MPO, PON1, and HDL may bind to each other, forming a ternary complex, wherein PON1 partially inhibits MPO activity and MPO inactivates PON1 influencing endogenous oxidative stress and lipid peroxidation during inflammation. In our previous study PON1 arylesterase activity was found to be an independent predictor of MPO levels in overweight hyperlipidemic, lipid-lowering therapy naive patients. In the nondiabetic obese group there were no significant correlations either between paraoxonase activity and MPO level or between obestatin and MPO level.

A previous study showed obestatin increased oxLDL binding to macrophages. Although, oxLDL level was significantly higher in obese patients, we could not find significant correlation between the levels of oxLDL and obestatin.

We concluded that decreased level of obestatin may contribute to the development of metabolic syndrome and altered lipoprotein metabolism in obese patients even without disturbed insulin sensitivity. However, obestatin level does not correlate to HDL function markers including PON1 and MPO and has no effect on the level of oxidized LDL. 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.

In our work, we provided new data about unfavorable effects of overweight and obesity by determining the quantitative and qualitative parameters of lipid metabolism. We have verified their correlations with the vascular biomarkers and the level of hormone protein produced by the digestive system, which can help to better understand the causes of increased cardiovascular risk associated to obesity and overweight. Thereby enabling new therapeutic pathways to be explored and widening future medication and non-medication treatment.

SUMMARY

Obesity is one of the leading causes of morbidity and mortality in the world and its prevalence has risen at an alarming rate over the past two decades. Numerous studies have shown a clear relationship between obesity and risk of developing cardiovascular disease (CVD). Dyslipidemia is frequently associated to obesity and overweight and a well-known risk factor of CVD. In dyslipidemia, the high-density lipoprotein (HDL) function is impaired characterized by increased levels of myeloperoxidase (MPO) and decreased paraoxonase-1 (PON1) activity, however, their relationships with other atherosclerotic biomarkers have not been completely clarified. Obestatin is a recently identified anorexigenic gut hormone. Its plasma concentrations were negatively correlated with body mass index (BMI) and insulin resistance index in obesity. Accumulating evidence supports its positive actions on both lipid metabolism and cardiovascular function. To date, level of obestatin and its correlations to the lipid subfractions in non-diabetic obese (NDO) patients have not been investigated.

Therefore, serum concentrations of lipid and inflammatory parameters, MPO levels and PON1 activities were investigated in 167 untreated hyperlipidemic overweight patients with and without vascular complications and in 32 healthy controls. Additionally, levels of CD40 ligand (sCD40L) and asymmetric dimethyl arginine (ADMA), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 and oxidized LDL (oxLDL) were determined. Furthermore, we aimed to measure the level of serum obestatin and evaluate its correlations to the lipid fractions and subfractions in non-diabetic obese (NDO) patients and in controls. We also investigated the possible associations between the concentration of obestatin and the HDL function characterized by PON1 acitivities and MPO levels.

In dyslipidemic overweight patients we found significantly higher glucose, hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride, lipoprotein(a), apolipoprotein B, C-reactive protein (CRP), MPO, sCD40L, sVCAM-1, sICAM-1 and oxLDL levels compared to the healthy subjects. We found elevated CRP, ADMA, sCD40L, sICAM-1 concentrations and higher MPO levels in patients with vascular complications compared to those without. PON1 arylesterase activity correlated negatively with sCD40L, ADMA and sICAM-1 levels, respectively. MPO concentrations showed positive correlations with sCD40L, ADMA, sICAM-1 levels, respectively. MPO concentration turned out to be best

predicted by sCD40L and ADMA levels in the whole study population and in patients without cardiovascular complications. However, in patients with vascular complications, only PON1 arylesterase activity was an independent (and negative) predictor of MPO level.

In NDO patients the serum level of obestatin was significantly lower 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 apolipoprotein A1, large HDL subfraction ratio and level, intermediate-density lipoprotein and mean LDL size. Serum very low-density lipoprotein (VLDL) ratio and level negatively correlated with obestatin. In multiple regression analysis obestatin was predicted only by VLDL level.

We conclude that in overweight dyslipidemic patients PON1 activity and MPO level correlate strongly with the vascular biomarkers, highlighting the importance of the HDL-associated pro- and antioxidant enzymes in the development of endothelial dysfunction and atherogenesis. 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.

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Registry number: DEENK/83/2018.PL
Subject: PhD Publikációs Lista

Candidate: Anita Szentpéteri
Neptun ID: YO8U0U
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Szentpéteri, A.**, Lőrincz, H., Somodi, S., Varga, V. E., Paragh, G. J., Seres, I., Paragh, G., Harangi, M.: Serum obestatin level strongly correlates with lipoprotein subfractions in non-diabetic obese patients.
   Lipids Health Dis. 17 (1), 1-27, 2018.
   DOI: http://dx.doi.org/10.1186/s12944-018-0691-y
   IF: 2.073 (2016)
2. **Szentpéteri, A.**, Zsíros, N., Varga, V. E., Lőrincz, H., Katkó, M., Seres, I., Fülöp, P., Paragh, G., Harangi, M.: Paraoxonase-1 and myeloperoxidase correlate with vascular biomarkers in overweight patients with newly diagnosed untreated hyperlipidaemia.
   VASA-J. Vasc. Dis. 46 (5), 370-376, 2017.
   DOI: http://dx.doi.org/10.1024/0301-1526/a000643
   IF: 1.242 (2016)

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List of other publications

3. Nádró, B., Juhász, L., **Szentpéteri, A.**, Páll, D., Paragh, G., Harangi, M.: Az apolipoprotein M és a szfingozin-1-foszfát tengely jelentősége az érelmeszesedés kialakulásának gátlásában. *Orvosi Hetilap. 159* (5), 168-175, 2018.
   DOI: http://dx.doi.org/10.1556/650.2018.30980
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4. Papp, C., Pák, K., Erdei, T., Juhász, B., Seres, I., **Szentpéteri, A.**, Kardos, L., Szilasi, M., Gesztelyi, R., Zsuga, J.: Alteration of the irisin-brain-derived neurotrophic factor axis contributes to disturbance of mood in COPD patients.
   Int. J. Chronic Obstr. Pulm. Dis. 12, 2023-2033, 2017.
   DOI: http://dx.doi.org/10.2147/COPD.S135701
   IF: 3.157 (2016)

5. Harangi, M., **Szentpéteri, A.**, Nádró, B., Lőrincz, H., Seres, I., Páll, D., Paragh, G.: HDL subfraction distribution and HDL function in untreated dyslipidemic patients.
   VP. 1, 166-173, 2017.
   DOI: http://dx.doi.org/10.20517/2574-1209.2017.27

6. Zsíros, N., Koncsos, P., Lőrincz, H., Seres, I., Katkó, M., **Szentpéteri, A.**, Varga, V. E., Fülöp, P., Paragh, G., Harangi, M.: Paraoxonase-1 arylesterase activity is an independent predictor of myeloperoxidase levels in overweight patients with or without cardiovascular complications.
   Clin. Biochem. 49 (12), 862-867, 2016.
   DOI: http://dx.doi.org/10.1016/j.clinbiochem.2016.03.011.
   IF: 2.434

Total IF of journals (all publications): 9,255
Total IF of journals (publications related to the dissertation): 3,315

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.

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