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Human paraoxonase-1 and adipokines in childhood obesity and lifestyle changes

by Péter Koncsos MD

Supervisor:
György Paragh MD, PhD, DSc

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Supervisor: György Paragh, MD, PhD, DSc

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
László Oláh, MD, PhD

The Examination takes place at the Conference Room in the Bldg. of the Faculty of Public Health, University of Debrecen
11 AM, February 24, 2014.

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

Reviewers:
Katalin Dankó, MD, PhD, DSc
László Barkai, MD, PhD, DSc

Members of the Defense Committee:
Anikó Somogyi, MD, PhD, DSc
László Oláh, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Clinical Center, University of Debrecen
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ABSTRACT

Childhood obesity is a predisposing factor for adult cardiovascular diseases. Human serum paraoxonase (PON1) may protect against atherosclerosis by hydrolyzing lipid peroxides in oxidized LDL. Alterations and potential correlations of PON1 activities, leptin and adiponectin levels in childhood obesity were studied. We investigated the effect of short-term lifestyle changes on the alteration of PON1 activities, leptin, adiponectin, E-selectin, asymmetric dimethylarginine (ADMA) as atherogenic and antiatherogenic factors in obese children and markers of endothelial dysfunction.

We measured PON1 paraoxonase and arylesterase activities, anthropometric parameters, leptin and adiponectin levels in 59 white, obese (age: 11,9±1,6 years) and 51 normal-weight children (age: 12,0±3,9 years). Twenty-three white obese and overweight children participated in a two-week long lifestyle camp based on diet and exercise program. Overweight and obesity were defined according to the national body mass index reference tables for age and gender.

Obese children had significantly lower PON1 paraoxonase and arylesterase activities, higher leptin and lower adiponectin levels compared to the normal-weight group. PON1 arylesterase activity showed inverse univariate correlation with leptin and positive correlation with adiponectin levels. In multiple regression analysis adiponectin was strongly associated with PON1 arylesterase activity in obese children. After two-week long supervised diet and aerobic exercise obese children had significantly decreased leptin, ADMA, E-selectin level, whereas they had significantly increased PON1 paraoxonase activity besides the antiatherogenic alteration of the lipid profile

and significant weight change. Adiponectin and PON1 arylesterase activity did not change significantly.

Our results emphasize the importance of the investigated metabolic alterations which may have further effects on cardiovascular morbidity and mortality in later adulthood. Altered levels of leptin, adiponectin and PON1 activities may be useful markers beside the general risk factors. Our investigations suggest that modifications in dietary habits and physical activity induce antiatherogenic changes in childhood obesity. These findings highlight the major role of primary prevention and non-pharmaceutical treatment of childhood obesity through lifestyle changes based on diet and increased physical activity.

INTRODUCTION

Childhood obesity

Adult and childhood obesity show increasing tendency worldwide. Obesity contributes to higher prevalence of cardiovascular diseases and other comorbidities as insulin resistance, type two diabetes mellitus, dyslipidemia, hypertension, chronic obstructive pulmonary disease, non-alcoholic fatty liver disease and malignancies. Childhood obesity also contributes to the higher prevalence of cardiovascular morbidity in the later adulthood.

Both genetic and environmental factors can lead to the expansion of adipose tissue and to the failure of energy homeostasis of the body. The disturbance of metabolic processes can result in the development of obesity through the increase of energy intake and/or the decrease of energy expenditure. Excessive food intake, lack of physical activity and psychological alterations belong to the environmental factors. The calculation of body mass index (BMI) is used for the determination of obesity in adulthood. Body mass index is defined as the individual's body mass divided by the square of height. BMI greater than 25 kg/m^2 is considered overweight and above 30 is considered obese. BMI percentile is used for the determination of childhood obesity that allows comparison with children of the same sex and age. Children above 90 percentile are considered to be overweight and above 97 percentile to be obese according to the Hungarian pediatric recommendations. Central obesity can contribute to the development of insulin resistance, type two diabetes mellitus, hypertension and coronary artery disease which is attributed to the expansion of the intraperitoneal adipose tissue and its adipokine profile.

The prevention of childhood obesity is regarded as an important issue because of its irreversible complications in adulthood. Prevention plays main role in management of childhood obesity which is based on lifestyle changes as diet and increased physical activity. Most of the pediatric protocols in childhood obesity recommend intervention above 95 BMI percentile and between 85-94 percentile with other comorbidities and positive family history.

Low-carbohydrate, low-fat, low-calorie and Mediterranean diet types are used during lifestyle interventions; diets should contain less energy needed for the maintenance of body weight but should not be less than 1200 kcal per day. Data regarding the efficacy of diet types are inconsistent in the literature. Regular physical activity and diet result in the improvement of insulin resistance, decrease in triglyceride, total cholesterol and LDL-C levels. Most of the investigations have shown increase in HDL-C levels and improvement in endothelial dysfunction in obesity. Pharmacological therapies and bariatric surgery could be the next steps after unsuccessful lifestyle interventions regarding the therapy of obesity; however, they are not recommended under the age of 18 in Hungary.

Adipokines

Adipose tissue is an active hormone-producing tissue besides its passive energy storage. These hormones are called adipokines which play regulatory role in the immune system, thrombotic processes and energy balance. The adipokine profile of the expanding visceral adipose tissue maintains a chronic inflammatory state which is responsible for the development of metabolic alterations and accelerated atherosclerosis in obesity.

Leptin

Leptin is a 16 kDa protein hormone which plays main role in the energy balance of the body. Leptin is produced mainly in expanding adipocytes after excessive energy intake; it affects the hypothalamic region by decreasing appetite and increases energy expenditure and fatty acid oxidation in peripheral tissues. Besides these effects, leptin plays role in immunological processes and sexual maturation in adolescents, too.

Obese adults and children have an increased level of leptin which shows positive correlation with the amount of body fat, BMI and other metabolic factors as triglyceride, LDL-C levels, blood pressure values, CRP levels and negative correlation with HDL-C levels. Elevated leptin levels are not only due to the increased amount of adipose tissue but also to leptin resistance in obesity. Increased leptin levels have atherogenic potential by stimulating the production of endothelin-1 in endothelial cells, the activity of inflammatory cells, migration of vascular smooth muscle cells and by increasing the production of reactive oxygen species (ROS) causing endothelial dysfunction. Human studies have shown that elevated leptin levels contribute to higher risk for cardiovascular diseases and stroke. Leptin levels show also gender difference which is more pronounced during and after sexual maturation.

Adiponectin

Adiponectin as an adipokine belongs to the complement 1q family expressed exclusively in the adipose tissue. Adiponectin plays main role in insulin sensitization; it stimulates fatty acid oxidation and glucose uptake in skeletal muscle cells and adipocytes, decreases glucose synthesis in the liver, decreases appetite and energy expenditure through its effects on the

hypothalamus. Adiponectin shows negative correlation with body fat mass; decreased levels can be found in adult and childhood obesity, patients with coronary artery disease and type two diabetes mellitus. This alteration is suggested to be due to the expanded visceral fat mass and chronic inflammatory state in obesity. Women have higher adiponectin level which is more pronounced during and after sexual maturation similarly to leptin levels.

Despite the effect of most adipokines, adiponectin has antiatherogenic effects: it correlates positively with apolipoprotein AI concentration, increases cholesterol efflux from macrophages, decreases the endothelial rolling of monocytes and the expression of proinflammatory cytokines.

Several studies have already investigated the effect of lifestyle changes on leptin and adiponectin levels both in adult and childhood obesity. The most pronounced decrease in leptin levels has been detected in patients with type two diabetes mellitus and during interventions with high degree of weight loss. Increase in adiponectin levels could be detected during interventions lasting more than two months and also with high degree of weight loss.

Antiatherogenic role of HDL

Atherosclerosis is considered to be a chronic inflammatory state that affects arteries and arterioles by hardening their wall through lipid accumulation which finally leads to obstruction or occlusion in these vessels. Atherosclerosis is the main cause of cardiovascular diseases as coronary artery disease, ischemic stroke and peripheral artery disease. The progressive obstruction of arteries is due to the lipid accumulation in macrophages and the proliferation of vascular smooth muscle cells which can finally contribute to acute vascular events through thrombotic processes. The development of so-

called fatty streaks as the first steps of atherosclerotic plaques can be detected even in childhood obesity. Endothelial dysfunction and oxidative modification LDL particles are considered to be the starting points of the chronic inflammatory process which may lead the accumulation of macrophages in the arterial wall and to the formation of atherosclerotic plaques. Visceral obesity, diabetes mellitus, dyslipidemia, smoking, hypertension, elder age, male gender and positive cardiovascular family history belong to the risk factors of atherosclerosis.

HDL-C levels show negative correlation with cardiovascular diseases which is attributed to different antiatherogenic effects of HDL: reverse cholesterol transport, direct endothelial and antioxidant effect in which the human paraoxonase-1 (PON1) plays an important role. The primary treatment goal of dylipidemia is the reduction of LDL-C levels; the increase of lower HDL-C levels as a secondary treatment goal can be managed through lifestyle changes, increased physical activity, weight loss, smoking cessation, decrease of dietary trans fats, increase of dietary omega-3 fatty acids or through pharmacological therapy with niacin or fibrate.

Human paraoxonase-1 and its antiatherogenic properties

Human paraoxonase-1 (PON1) belongs to the paraoxonase gene family which is produced mainly in the liver and bound to HDL particles. The enzyme is named after the paraoxone molecule, an active metabolite of the insecticide parathione which can be hydrolysed by PON1. The enzyme has both lactonase and esterase activities but the physiological substrate has been unknown so far. PON1 has antiatherogenic effect by hydrolysing lipid-peroxides in LDL and HDL particles which has been proven also in human studies. Decreased

PON1 activities have been measured in diabetes mellitus, adult obesity and chronic kidney disease. The activity of the enzyme is affected by both genetic and environmental factors and measured with paraoxone and phenylacetate substrate which correlates well with the serum level of PON1.

LDL particles show increased resistance against oxidative stress in regular physical activity; however, studies investigating the effect of lifestyle changes on the level and activity of PON1 have been inconsistent so far. Although none of these studies have investigated the direct causes of the increase in PON1 activity, it is suggested that regular physical activity enhances the endogenous antioxidant system of the body and in this way the rate of the enzyme inactivation by oxidative stress also decreases resulting in higher PON1 activity.

Role of asymmetric dimethylarginine in endothelial dysfunction

The first important step in the process of atherosclerosis is the development of endothelial dysfunction which is due the imbalance of vasoactive agents. Nitric oxide, (NO) which is produced by the endothelial NO-synthase (eNOS), results in vasodilation by signaling the surrounding smooth muscle cells to relax. Asymmetric dimethylarginine (ADMA) is a methylated L-arginine derivative, a metabolic degradation by-product of modified proteins. ADMA is an endogenous inhibitor of eNOS; it can inhibit the production of NO. Elevated levels of ADMA show strong positive correlation with cardiovascular diseases in human studies. Both obese adults and children have increased levels of ADMA whereas physical activity results in significant decrease of ADMA levels in adult patients with metabolic syndrome.

E-selectin levels as one of the markers of vascular inflammation are also elevated in obese children. Lifestyle changes decrease significantly the levels of E-selectin along with the decrease of oxidative markers.

AIMS

The effect of childhood obesity on PON1 activity, leptin and adiponectin levels

Prevoius studies have found elevated leptin and lower adiponectin levels both in adult and childhood obesity. PON1 showed decreased activity in adult obesity; however, the effect of childhood obesity on PON1 activity has not been investigated yet. PON1 activity showed significant negative correlation with leptin and positive correlation with adiponectin levels. Several causes have been suggested so far: leptin as a hydrophobic protein is able to bind to HDL particles inhibiting directly the PON1 enzyme, enhances oxidative stress and the production of inflammatory cytokines which contribute to the inhibition of PON1 synthesis. On the other hand, leptin increases the expression of serum amyloid A protein which is able to replace apolipoprotein AI in HDL that plays main role in the structural stabilization of PON1. Adiponectin has antiatherogenic effects by enhancing reverse cholesterol transport, apoAI mediated cholesterol efflux from the cells by increasing the production of HDL particles.

The effect of lifestyle changes on PON1 activity, adipokine, E-selectin and ADMA levels

Based on these observations, we hypothesized that PON1 activity may be lower in childhood obesity compared to normal-weight children and there may

be a significant relation between PON1 activity and adipokine levels. We also investigated the effect of short-term lifestyle changes on decreased PON1 activity in childhood obesity since it had not been investigated before. Previous studies found significant changes in adipokine levels during long-term interventions with high degree of weight loss.

We measured E-selectin and ADMA levels in order to determine indirectly the changes of endothelial dysfunction because these parameters show strong positive correlation with the degree of oxidative stress. E-selectin showed significant reduction after two-week long lifestyle-modification in obese children in a previous study; however, the effect of physical activity and diet had not been investigated in childhood obesity yet.

We hypothesized that two-week long lifestyle modification induces significant increase in PON1 activity, adiponectin levels and decrease in leptin levels along with the improvement of insulin resistance, anthropometric parameters and lipid values.

METHODS

Patients

We investigated the effect of childhood obesity on PON1 activity and adipokine levels in 59 Caucasian overweight, obese (obese group: OB; average age: 11,95±1,61 years; 25 girls, 34 boys) and 51 Caucasian normal-weight (control group, C; average age: 12,00±3,91 years; 22 girls, 29 boys) children. 23 overweight, obese children (average age: 11,57±1,78 years; 8 girls, 15 boys) took part in a two-week long lifestyle camp in order to investigate the effect of lifestyle changes on the above mentioned parameters, E-selectin and ADMA levels. We used the Hungarian BMI percentile curve to determine obesity: children above 90 percentile belonged to the overweight-obese group (OB: 95,08±3,53% vs. C: 64,10±8,36%). The normal-weight children were recruited from the 3rd pediatric practice of Hajdúböszörmény with the voluntary consent of both the children and their parents matched in gender and age to the overweight group. None of the normal-weight and overweight children had any chronic diseases (diabetes mellitus, endocrinological disorders, hereditary diseases or systemic inflammation) or was taking any medications. Participants of the study belonged to the Tanner stage I-IV, defined on the basis of breast development in girls and genital development in boys.

Lifestyle camp

The obese children took part in a two-week long life-style program. Reduction of body weight was achieved during 14 days in a lifestyle camp using both decrease in food intake and enhanced energy expenditure: 500-meter run (13 MET hour) twice a day, 1000 meters swimming (8 MET hour) every second

day, 2,5 km walking (3 MET hour), indoor and outdoor ball games (7 MET hour). Obese children spent an average total time of 3,5-4,5 hours a day with exercises. Children were on low-calorie diet with five meals a day. Prepared meals contained 1200-1500 kilocalories/day based on the individual age and qualitatively 20-25% of calories from fat, 25-30% of calories from protein and 50-55% of calories from unrefined carbohydrate, high in dietary fiber. Fatness indices as BMI, BMIA, waist circumference, body fat percentage (BFP) were also determined. BFP was measured with bioelectrical impedance analysis (BIA, Biodynamics, Model 310, Seattle, WA). Systolic and diastolic blood pressures were measured three times with the subject in sitting position after resting for at least 5 minutes using a quality-approved automatic electronic sphygmomanometer.The research protocol was approved by the Ethics Committee of the University of Debrecen, Hungary, Medical and Health Science Center. Informed written consent was obtained from all parents and oral consent from all children.

Blood sampling

After overnight fasting, 10 ml of venous blood was drawn between 08:00h and 09:00h on the 1st and the 15th day of the lifestyle camp. Hemoglobin, hematocrit, white blood cell count, sedimentation rate, liver enzymes, urea, creatinine, creatine kinase, bilirubin, serum glucose, cholesterol, HDL-C, LDL-C, triglyceride, insulin, C-reactive protein (CRP) were determined from fresh serum. The sera for leptin, adiponectin, E-selectin, ADMA levels, PON1 paraoxonase and arylesterase activity measurements were kept at -70°C before analysis.

Evaluation of adipokines, PON1 paraoxonase and arylesterase activities, E-selectin, ADMA, insulin levels and lipid parameters

Serum leptin (BioVendor Laboratory Medicine, Inc.; Czech Republic), adiponectin and E-selectin (R&D Systems, Inc.; USA) levels were measured by sandwich enzyme immunoassays. Serum ADMA levels were measured by competitive enzyme immunoassays (DLD Diagnostika GmBH, Hamburg, Germany). Paraoxonase activity of the PON1 enzyme was determined using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma) as substrate and measured by the increase in the absorbance at 412 nm due to the formation of 4-nitrophenol. The activity was measured at 25 ^{o}C, by adding 50 μl of serum to 1 ml Tris/HCl buffer (100 mmol/l, pH 8,0) containing 2 mmol/l $CaCl_2$ and 5,5 mmol/l paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm using a spectrophotometer. Enzymatic activity was calculated from the molar extinction coefficient 17100 M^{-1} cm^{-1}. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above described assay conditions. Arylesterase activity of the PON1 enzyme was measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris/HCl pH 8,0. The reaction was started by the addition of the serum and the absorbance increase was determined at 270 nm as previously described. Enzyme activity was calculated using a molar extinction coefficient of 1310 $M^{-1}cm^{-1}$. 1 unit (U) is defined as 1 mmol phenylacetate hydrolyzed per minute.

Serum cholesterol and triglyceride levels were measured by using enzymatic colorimetric tests (GPO-PAP, Modular P-800 Analyzer, Roche/Hitachi), while HDL-C was assessed by a homogenous enzymatic colorimetric assay (Roche HDL-C plus 3rd generation). The LDL-C fraction was calculated indirectly

using the Friedewald equation (triglyceride level < 4,5 mmol/l). CRP levels were measured with latex-based high-sensitive immunoturbidimetric method (Cobas Integra-800, Roche Ltd, Mannheim, Germany). The serum concentration of insulin was measured by a commercially available radioimmunoassay kit (MP Biomedicas, Orangeburg, NY). HOMA (homeostasis model assessment) scores were calculated using the formula: fasting insulin (μU/ml) * fasting glucose (mmol/l) / 22,5.

Statistical methods

The statistical analysis was performed by SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented by descriptive analysis (case number, mean, standard deviation; in case of non-normal distribution median, lower and upper quartile). Comparisons between groups were performed by Student t-test. Non-normally distributed parameters as leptin, adiponectin levels, PON1 paraoxonase and arylesterase activities were transformed logarithmically to correct their skewed distributions. Relationships between parameters were assessed by Pearson correlation analysis. We carried out multiple regression analysis using the stepwise method to determine the variables best predicted PON1 arylesterase activities adjusting for age, sex, BFP, HDL-C, leptin, adiponectin in the model. Comparisons before and after the lifestyle camp were performed with Student's paired t-test. Distributions of data were tested with Kolmogorov-Smirnov test; all of the presented data had normal distribution. Relationships between parameters and their changes after the lifestyle-program were assessed by Pearson-correlation analysis. We used two-sided p-values; $p<0,05$ probability was accepted as the level of significance.

RESULTS

Comparisons between the parameters of obese and normal-weight children

There was no difference in age, sex ratio and Tanner stage between the two groups. HDL-C levels (OB: 1,12±0,26 mmol/l vs. C: 1,27±0,28 mmol/l, $p<0,05$) were significantly lower in obese children and they had higher total and LDL-C, triglyceride (TG) and fasting glucose levels but none of these differences were significant compared to the control group. Regarding the insulin-resistance status, obese children had significantly higher HOMA-IR (OB: 8,40±3,64 vs. C: 1,17±0,44, $p<0,0001$). There was not any significant difference in blood pressure values. 5 children with higher LDL-C (≥3,4 mmol/l) levels, 17 with lower HDL-C levels (≤1,0 mmol/l) and 10 with higher triglyceride levels (≥1,7 mmol/l) could be detected in the obese group according to the IDF recommendation. None of the children had blood glucose level above 5,6 mmol/l.

PON1 acitivities and adipokine levels in childhood obesity

Obese children had significantly lower PON1 paraoxonase and arylesterase activities (PON1 arylesterase activity OB: 94,40(82,20/108,70) U/l vs. C: 115,20(93,70/126,00) U/l, $p<0,01$; PON1 paraoxonase activity OB: 84,80(64,33/144,74) U/l vs. C: 99,42(83,33/152,05) U/l, $p<0,05$), lower adiponectin levels (OB: 7,56(5,69/12,06) μg/ml vs. C: 11,51(8,84/14,49) μg/ml, $p<0,001$) and higher leptin levels (OB: 37,05(24,33/53,87) ng/ml vs. C: 4,62(2,52/17,6) ng/ml, $p<0,0001$) compared to the normal-weight children.

Although girls had a higher tendency in both adipokine levels, it did not prove to be significant in statistical analysis.

Correlations between the parameters of the obese group

To investigate the relation of the above mentioned parameters to each other, we carried out Pearson correlation analysis. Although both activities of PON1 were significantly lower in the obese group compared to the normal-weight group, only PON1 arylesterase activity showed significant positive correlation with adiponectin ($r=0,39$, $p<0,01$) and negative correlation leptin levels ($r=-0,29$, $p<0,05$). Leptin levels showed also significant correlation with anthropometric parameters as BMI, BFP, waist circumference, systolic and diastolic blood pressure values similarly to the results of other previous studies. HOMA-IR values of the obese group showed significant positive correlation with leptin levels ($r=0,42$, $p<0,01$) and negative correlation with adiponectin levels ($r=-0,47$, $p<0,01$). We found also significant correlation between adiponectin and HDL-C levels of the obese group ($r=0,33$, $p<0,05$).

To test whether the associations of PON1 arylesterase with leptin and adiponectin in the univariate analysis were independent of other parameters, we carried out multiple regression analysis using stepwise method. In the first model, we investigated the two adipokines, which showed significant univariate correlation with PON1 arylesterase activity, and the impact of age and sex. In this model, adiponectin showed strong association with PON1 arylesterase activity ($\beta=0,45$, $p<0,004$). Although HDL-C and BFP did not show significant univariate correlation with PON1 arylesterase activity, we investigated their impact in the second model adjusting them to the previous parameters, since PON1 is associated to a subfraction of HDL and BFP reflects well the degree of obesity in adolescent children. Adiponectin proved

to be also a significant predictor of PON1 arylesterase activity in this regression model (2nd model: β=0,45, p<0,02).

The effect of lifestyle changes on the anthropometric and clinical data of the obese children

There was significant decrease in BMI, BFP and waist circumference as anthropometric data. We could detect significant decrease in systolic and diastolic blood pressure. Regarding the lipid profile, total cholesterol, LDL-C and triglyceride levels decreased significantly while HDL-C levels did not show significant alteration. Insulin and glucose levels along with HOMA-IR values were markedly decreased after the lifestyle camp. Significantly improved physical performance was measured after the lifestyle camp in box-stepping exercise in 30 seconds (22,09±3,05 vs. 26,95±5,38; p<0,0001), push-ups in 30 seconds (10,68±2,71 vs. 12,23±2,56; p<0,01), 200-meter run (62,30±13,29 s vs. 57,48±8,16 s; p<0,01) and sit-up exercise (14,81±4,65 vs. 17,52±4,76; p<0,001).

The effect of lifestyle changes on leptin, adiponectin, E-selectin, ADMA levels and PON1 activities in obese children

Two-week long physical activity and diet resulted in significant decrease in leptin levels (55,02±33,42 ng/ml vs. 25,37±19,07 ng/ml, p<0,0001); however, changes in adiponectin levels did not prove to be significant. E-selectin (67,19±30,35 ng/ml vs. 46,51±18,40 ng/ml, p<0,0001) and ADMA levels (0,68±0,15 μmol/l vs. 0,55±0,16 μmol/l, p<0,01) showed also significant reduction. Decrease in CRP levels as the marker of chronic inflammation was not significant. We detected significant increase only in PON1 paraoxonase

activity (110,48±72,92 U/l vs. 121,75±93,48 U/l, $p<0,05$) which reflects well the influence of environmental factors as oxidative stress on the enzyme. The change in PON1 arylesterase activity, which correlates well with the hepatic production of the PON1 enzyme, was not significant after the lifestyle intervention along with the change in adiponectin levels that proved to be an independent predictor of arylesterase activity in the previous investigation.

We found significant positive correlation of leptin concentration with BMI ($r=0,472$; $p<0,05$), BFP ($r=0,75$; $p<0,001$), HOMA ($r=0,524$; $p<0,05$) and triglyceride levels ($r=0,515$; $p<0,05$) with Pearson-correlation analysis before the lifestyle program similarly to the previous part of our study. Adiponectin showed significant positive correlation with HDL-C levels ($r=0,635$; $p<0,01$) and significant negative correlation with HOMA values ($r=-0,46$; $p<0,05$). Changes of the investigated parameters did not show any significant correlations with each other that could be due to the lower number of the subjects.

DISCUSSION

Previous studies found lower PON1 acitivities in adult obesity; however, PON1 activity and its relationship with leptin and adiponectin levels have not been investigated in childhood obesity yet. Previous investigations found increased leptin and decreased adiponectin levels both in adult and childhood obesity similarly to our results. Obese children had significantly lower PON1 paraoxonase and arylesterase activities compared to their normal-weight peers whereas PON1 arylesterase activity showed significant correlation with both of the investigated adipokines. Although PON1 paraoxonase activity did not correlate with these adipokines, it must be noted that paraoxonase activity has high interindividual variability. Since arylesterase activity correlates well with the serum level of PON1, it is suggested that leptin and adiponectin may have influence on the production of PON1. To test whether the associations of PON1 arylesterase with leptin and adiponectin were independent of other parameters, we carried out multiple regression analysis: adiponectin proved to be independent predictor of arylesterase activity similarly to another study with adult obese patients. This finding may be supported by the fact that adiponectin enhances reverse cholesterol transport, apoAI-mediated cholesterol efflux through the increase of HDL production; adiponectin shows strong correlation with HDL-C levels similarly to our results. The inverse relation of leptin with PON1 arylesterase activity may be explained with the increased enzyme-inactivation by oxidative stress and with the inhibition of the hepatic production of PON1 due to the enhancement of acute phase reaction. Leptin showed also significant positive correlation with BMI, BFP, with the degree of insulin resistance as HOMA-IR and other metabolic parameters as triglyceride levels, diastolic and systolic blood pressure values

similarly to the results of other previous studies. Although these studies found significant correlation between adipoonectin and anthropometric parameters, we could not prove this finding in our results but adiponectin showed significant correlation with leptin levels, HOMA-IR and HDL-C. PON1 activity showed decreasing tendency with ageing in a previous study; it suggests that decreased PON1 activity in childhood obesity may contribute to increased cardiovascular morbidity in later adulthood. Other investigations found gender differences in both adipokines; we detected higher tendency in leptin and adiponectin levels of the obese girls but it did not prove to be statistically significant. This result may be due to the average age of the obese group which was about 12 years and to the fact that the children belonged to different Tanner stages; gender differences in adipokine levels have been observed mainly during and after sexual maturation.

We found atherogenic alteration of PON1 activity, leptin and adiponectin levels in childhood obesity; in the other part of our study, we investigated the effect of lifestyle changes whether it could influence the above mentioned parameters. Leptin levels showed significant reduction along with the decrease of BMI, BFP, HOMA-IR, waist-circumference that showed significant correlation with leptin levels similarly to other studies. Overweight children showed also significantly improved physical performance after the lifestyle camp. We did not find any significant change in adiponectin levels similarly to previous studies that found significant increase mainly after more than two-month long interventions resulting in significant weight loss. We found significant increase only in PON1 paraoxonase activity; the change in arylesterase activity was not significant. Although the direct causes of this finding have not been elucidated yet, several factors are suggested to be

related with it. It is known that increased physical activity enhances the expression of antioxidant genes, the antioxidant protection of the body and decreases lipid peroxidation. Elevated leptin levels increase the production of reactive oxygene species in endothelial cells that may contribute to the inactivation of PON1 and may explain lower PON1 paraoxonase activity and decreased expression of the enzyme through the enhancement of the acute phase reaction. The decrease of these inhibitory effects may contribute to the elevation of lower PON1 paraoxonase activity in childhood obesity; this type of activity reflects well the effect of environmental factors on the enzyme. Since the increase in PON1 arylesterase activity and adiponectin levels did not prove to be significant, it is suggested that there may not have been any significant changes in the production of the enzyme, too. In the first part of our study, adiponectin proved to be an indepenedent predictor of arylesterase activity similarly to the results of adult obese population. Adiponectin may influence the hepatic expression of PON1 through its above mentioned effects on the production of apoAI and HDL. CRP levels, that reflect well the degree of chronic inflammation in obesity and may also influence the production of PON1, did not show any significant change during the intervention, too. Based on these data, it is suggested that short-term lifestyle changes may not influence significantly the production of PON1 but may result in significant decrease of the oxidative stress that is reflected well by the paraoxonase activity of PON1. This finding is also supported by a previous study that found significant decrease in 8-iso prostaglandin-F2α and E-selectin levels as oxidative and endothelial markers after two-week long physical activity and diet in case of obese children.

Both E-selectin and ADMA levels showed significant reduction after the lifestyle intervention that suggests the improvement of endothelial dysfunction; this result may be due to the increase of PON1 activity, the decrease of oxidative stress generated by lower leptin levels and to the reduction of ADMA levels. We found significant decrease both in diastolic and systolic blood pressure values; however, they did not show any correlation with ADMA levels which may be due to the low number of subjects.

Other studies have inconsistent results regarding HDL-C levels during lifestyle interventions. We found non-significant HDL-C decrease which may have been due to the effect of low-fat diet. We found significant decrease in total, LDL-cholesterol and remarkable reduction in triglyceride levels which may have been due to the effect of the diet and on the other hand to the increased physical activity that results in enhanced lipolysis, decrease in VLDL synthesis through the improvement of insulin resistance and increased VLDL-clearence by the muscles.

We did not find any significant correlation between PON1 paraoxonase activity and other parameters; however, it must be noted that paraoxonase activity has high interindividual variability and there was a low number of subjects during the intervention.

Conclusion

Previous studies have already proven that the antioxidant activity of PON1 decreases with ageing; in this way, lower PON1 activity along with the atherogenic alteration of adipokines in childhood obesity may contribute to the increase of cardiovascular diseases in later adulthood. It is suggested that even

short-term lifestyle interventions with physical activity and diet may induce antiatherogenic changes in PON1 activity, adipokine, lipid levels and result in the improvement of endothelial dysfunction through the alteration of E-selectin and ADMA levels. PON1 activity, leptin and adiponectin levels may be early atherogenic markers besides the regular clinical and laboratory parameters in childhood obesity. These findings emphasize the major role of primary prevention and treatment of childhood obesity through lifestyle changes based on diet and increased physical activity.

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List of publications related to the dissertation

1. **Koncsos, P.**, Seres, I., Harangi, M., Páll, D., Józsa, L., Bajnok, L., Nagy, E., Paragh, G.: Favorable effect of short-term lifestyle intervention on human paraoxonase-1 activity and adipokine levels in childhood obesity.
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