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Paraoxonase-1 Activity in Overweight and Obese Children and Adolescents: Association with Obesity-Related Inflammation and Oxidative Stress

Aktywność paraoksonazy-1 u dzieci i nastolatków z otyłością i nadwagą: związek ze stanem zapalnym i stresem oksydacyjnym

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Abstract

Background. Paraoxonase-1 (PON1) is a HDL-attached extracellular esterase which is believed to contribute to the anti-atherogenic and anti-inflammatory properties of HDL. A decrease in PON1 is a risk factor for cardiovascular disease and has recently been found to be associated with juvenile obesity. The issue of a possible association between enzyme activity and/or its phenotype distribution and obesity-related metabolic abnormalities, inflammation, and oxidative stress has not been addressed yet.
Objectives. To evaluate PON1 activity and phenotype distribution with respect to obesity and obesity-related metabolic disorders, inflammation and oxidative stress in children and adolescents.
Material and Methods. PON1 arylesterase activity was measured spectrophotometrically in 156 children and adolescents (47 lean, 27 overweight and 82 obese). Enzyme phenotype was determined using dual substrate (phenyl acetate/paraoxon) method. PON1 activity and phenotype distribution were related to the presence of obesity, metabolic syndrome, insulin resistance, hyperinsulinemia, hypertriglyceridemia, high blood pressure, low HDL level, impaired fasting glucose and/or glucose tolerance as well as inflammatory and oxidative stress indices.
Results. PON1 arylesterase activity decreased in general and central obesity, high blood pressure, and hyperinsulinemia conditions and correlated with BMI, CRP, adipocyte fatty acid-binding protein, superoxide dismutase, catalase, glutathione peroxidase, free thiols, and HOMA in a gender-dependent manner. PON1 decreases were independently associated with central obesity in girls, explaining 17% in PON1 variability, and with elevated CRP in boys, explaining 12% in its variability. PON1 phenotype was not associated with frequency of metabolic abnormalities.
Conclusions. PON1 decreases in central obesity, exacerbating obesity-related inflammation and oxidative stress. The enzyme associations are gender-dependent: obesity and oxidative stress affects PON1 in girls whereas inflammation in boys (**Adv Clin Exp Med 2013, 22, 2, 229–236**).

Key words: paraoxonase-1 (PON1), obesity, metabolic syndrome, children, inflammation, oxidative stress.

Streszczenie

Wprowadzenie. Paraoksonaza-1 (PON1) to zewnątrzkomórkowa esteraza związana z cząsteczkami HDL, która odpowiada za ich antyaterogenne i antyzapalne właściwości. Zmniejszenie aktywności PON1 jest czynnikiem ryzyka rozwoju chorób sercowo-naczyniowych. Ostatnie badania wskazują na związek między zmniejszeniem aktywności enzymu a otyłością u dzieci i młodzieży. Brakuje jednak danych literaturowych dotyczących aktywności i fenotypów enzymu w zaburzeniach metabolicznych, stanie zapalnym i stresie oksydacyjnym towarzyszących otyłości w tej grupie wiekowej.
Cel pracy. Ocena aktywności i rozkładu fenotypów PON1 w otyłości dziecięcej oraz związanych z otyłością zaburzeniach metabolicznych, stanie zapalnym i stresie oksydacyjnym.
Materiał i metody. Spektrofotometrycznie zmierzono aktywność arylesterazową PON1 u 156 dzieci i nastolatków (47 szczupłych, 27 z nadwagą i 82 otyłych). Fenotyp enzymu określano metodą dwóch substratów względem octanu fenylu i paraoksonu. Aktywność enzymu i rozkład fenotypów odniesiono do występowania otyłości, zespołu metabolicznego, insulinooporności, hiperinsulinizmu, hipertriglycerydemii, wysokiego ciśnienia, małego stężenia HDL, nieprawidłowej glikemii na czczo i/lub nietolerancji glukozy, a także stanu zapalnego i stresu oksydacyjnego.
Wyniki. Aktywność PON1 była zmniejszona w otyłości ogólnej i brzusznej, przy wysokim ciśnieniu i hiperinsulinemii. Aktywność PON1 korelowała z BMI, CRP, białkiem wiążącym kwasy tłuszczowe pochodzącym z adipocytów, dysmutazą nadtlenkową, katalazą, peroksydazą glutationową, wolnymi grupami sulfhydrylowymi oraz wskaźnikiem HOMA w sposób zależny od płci. U dziewcząt otyłość brzuszna była niezależnym czynnikiem predykcyjnym aktywności PON1, wyjaśniając 17% jej zmienności. U chłopców stężenie CRP wyjaśniało 12% zmienności w aktywności PON1. Nie wykazano związku między fenotypem PON1 a występowaniem zaburzeń metabolicznych.
Wnioski. Aktywność PON1 jest zmniejszona w otyłości brzusznej u dzieci, przyczyniając się do zaostrzenia współistniejącego stanu zapalnego i stresu oksydacyjnego. Otyłość wpływa na aktywność enzymu u dziewcząt, a u chłopców na stan zapalny (**Adv Clin Exp Med 2013, 22, 2, 229–236**).

Słowa kluczowe: paraoksonaza-1 (PON1), otyłość, zespół metaboliczny, dzieci, stan zapalny, stres oksydacyjny.

Paraoxonase-1 (PON1) [EC 3.1.8.1] is a HDL-attached, extracellular esterase synthesized mainly in the liver. PON1 is believed to contribute to the anti-atherogenic and anti-inflammatory properties of HDL; it degrades lipid peroxides, decreases HDL susceptibility to peroxidation, glycation, and homocysteinylation, and increases cholesterol efflux from macrophages [1]. In animal models of obesity and the metabolic syndrome, serum activity of PON1 is diminished. The human PON1 gene presence, in turn, restores the enzyme activity and is associated with decreased plaque volume and number of macrophages, as well as lipid peroxides [1]. Accordingly, in human obesity PON1 activity decreased and correlated with increases in lipid hydroperoxides in both HDLs and LDLs [2]. PON1 has also been found an independent risk factor for cardiovascular disease in some [3] but not all studies [4].

PON1 phenotype is believed to reflect PON1 association with atherosclerotic risk better than its genotype [3]. A number of PON1 polymorphisms has been described, with Q192R most spectacularly affecting the enzyme's activity. PON1 in individuals homozygous for the Q alloenzyme (A phenotype of PON1 with Q at 192) has greater antioxidant properties while PON1 in heterozygous (AB phenotype of PON1) and homozygous for the R alloenzymes individuals (B phenotype of PON1 with R at 192) has diminished antioxidant properties [1].

Juvenile population is relatively free of potential confounders and the metabolic abnormalities, risk factors for cardiovascular disease, occur already in childhood. Yet, their association with PON1 activity in children and adolescents has been limited to only one study concerning obesity [5]. The issue of possible association between PON1 arylesterase activity and/or its phenotype distribution and obesity-related metabolic abnormalities, inflammation or oxidative stress have not been addressed yet.

Material and Methods

This study is derived from research on oxidative stress and inflammation in Polish overweight/obese children and adolescents [6–10] conducted by the Department of Endocrinology and Diabetology of Children and Adolescents in cooperation with Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland. Within the original cohort (n = 167), blood samples were available for PON1 evaluation for 156 children and adolescents (age 10–17 years): 109 individuals treated for metabolic disorders associated with primary overweight (n = 27) or obesity (n = 82) and 47 controls, who where lean (BMI < 85th percentile, normal body build), age- and gender-matched, apparently healthy (no visible signs of any illness and unremarkable medical history, CRP within reference range). The control group was recruited from hospital staff children and students from regional secondary school. Overweight/obese patients with hypothalamic obesity, genetic disorders, Cushing's

syndrome, growth hormone deficiency, insulinoma, hypothyroidism, and iatrogenic obesity were not included. Blood samples were collected following a complete physical examination of all participants, encompassing the evaluation of anthropometric parameters (measured to the nearest 0.1 kg/0.1 cm), blood pressure and Tanner staging. An oral glucose tolerance test was performed (1.75 g of glucose/1 kg; glucose/insulin measured at baseline-1h-2h) in overweight/obese subjects. Detailed characteristics of study population are presented in Table 1.

Definitions

Overweight and obesity were defined according to the International Obesity Task Force (IOTF) criteria [11]. BMI was calculated as weight/height squared [kg/m^2], referred to the gender- and age-dependent median BMI values devised for Polish population [12]. BMI was normalized: BMI% = 100×log$_e$(BMI/median BMI), as an optimal measure of weight changes in growing children [13]. Central obesity was defined as waist circumference (WC)≥90th percentile for age and gender according to national centile charts [14]; hyperglycemia was identified when fasting glucose ≥ 5.55 mmol/L; hyperinsulinemia when fasting insulin ≥ 104.18 pmol/L, or oral glucose tolerance test insulin ≥ 1041.8 pmol/L and/or ≥ 520.88 pmol/L at 2h; insulin-resistance when homeostasis model assessment (HOMA) > 2.7 [15]. Diastolic and/or systolic BP > 90th percentile for age, gender, and height was considered high [16]. Metabolic syndrome was recognized in 52 individuals when at least three of the following criteria were met: fasting glucose ≥ 5.55 mmol/L, triglycerides ≥ 1.24 mmol/L, HDL-C < 1.036 mmol/L, WC > 90th percentile for age and gender, blood pressure > 90th percentile for age, gender, and height [17].

Ethical Considerations

The study protocol was approved by the Medical Ethics Committee of Medical University and was in accordance with the ethical standards stated in the Helsinki Declaration of 1975. Informed consent was obtained from the study participants and their parents.

Analytical Methods

Sera were collected following a 12-hour fast, centrifuged (15 min., 720×g); whole blood was col-

Table 1. Baseline demographic and metabolic characteristics of study population stratified by weight status

Tabela 1. Charaterystyka demograficzna oraz profil metaboliczny badanej populacji z uwzględnieniem kategorii wagowej

	Lean (Prawidłowa waga) n = 47	Overweight (Nadwaga) n = 27	Obese (Otyłość) n = 82	P value
Gender, F/M (Płeć – K/M)	23/24	16/11	48/34	0.528
Age, yrs (Wiek – lata)	14.5 ± 2.1	14.7 ± 1.8	14.3 ± 1.9	0.623
Puberty, I/II–IV/V (Dojrzałość płciowa)	2/28/17	0/17/10	7/44/31	0.519
BMI, kg/m^2	19.2 ± 2.4	26.9 ± 1.5*	32.4 ± 3.5*,†	< 0.001
FG, mmol/L	5.22 ± 0.6	5.29 ± 0.8	5.27 ± 0.7	0.910
FI, pmol/L	62.2 ± 41	79.4 ± 42	113.4 ± 61*,†	< 0.001
HOMA	2.05 ± 1.5	2.68 ± 1.5	3.83 ± 2.4*,†	< 0.001
tCHOL, mmol/L	4.51 ± 0.8	4.45 ± 0.9	4.68 ± 0.8	0.359
HDL-C, mmol/L	1.54 ± 0.33	1.42 ± 0.28	1.30 ± 0.27*	< 0.001
LDL-C, mmol/L	2.55 ± 0.69	2.47 ± 0.76	2.71 ± 0.71	0.254
TG, mmol/L	0.96 ± 0.32	1.22 ± 0.53	1.44 ± 0.66*	< 0.001

* significantly different from lean; † significantly different from overweight. F/M – female to male ratio; BMI – body mass index; FG – fasting glucose; FI – fasting insulin; HOMA – homeostasis model assessment; tCHOL – total cholesterol; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein; TG – triglycerides.

* znamiennie różne od prawidłowej wagi; † znamiennie różne od nadwagi. K/M – stosunek kobiet do mężczyzn; BMI – wskaźnik masy ciała; Glu – glukoza na czczo; Ins – insulina na czczo; HOMA – wskaźnik insulinooporności; cCHOL – całkowity cholesterol; HDL-C – cholesterol HDL; LDL-C – cholesterol LDL; TG – triglicerydy.

lected into citrate-tubes and centrifuged (15 minutes, 720×g). Serum and erythrocytes were stored at –80°C until analysis. Prior to the analysis erythrocytes were hemolyzed by dissolving in cold water (1:60 (v/v)) and 15' incubation on ice. Serum PON1 activity was measured as its arylesterase activity – a rate of phenyl acetate hydrolysis (Sigma-Aldrich, St. Louis, MO), according to the Arylesterase/Paraoxonase assay kit protocol (ZeptoMetrix Co., Buffalo, NY) (intra-assay CV = 3.0%). One unit [U] of enzyme activity was defined as one mmol of released phenol/liter of serum/minute. Arylesterase activity, not being affected by common PON1 polymorphisms, has been demonstrated to correspond with enzyme concentration [18]. Dual substrate method [18] was used for PON1 phenotyping: plotting arylesterase activity against paraoxonase activity resulted in separation of two forms of PON1: with low and high paraoxonase activity. The former represents individuals with A phenotype of PON1 and the latter represents individuals with AB and B phenotype combined. Paraoxonase activity of PON1 was determined as rates of paraoxon hydrolysis (ChemService Inc., West Chester, PA) with Charlton-Menys et al. method [19] (intra-assay CV = 1.1%). One unit [U] of enzyme activity was defined as one μmol of released p-nitrophenol/liter of serum/minute. CRP was measured by a highly sensitive immunonefelometric assay using CardioPhase HS-CRP (Dade Behring Marburg GmBH; Marburg, Germany) on BN II Dade Behring analyzer. The activities of copper-zinc superoxide dismutase (SOD1), glutathione peroxidase-1 (GPx1), and catalase (CAT) were assessed in erythrocyte lysates. They were expressed in units of activity [U] and standardized to avoid possible artificial differences caused by sample preparation by calculating activity per unit of hemoglobin present in hemolysate [U/unit of hemoglobin]. Hemoglobin concentration in the hemolysate was assessed spectrophotometrically through the evaluation of hemolyzed erythrocyte samples at 578 nm in the solution of 0.1% ammonia. SOD1 and GPx1 were assessed using RANSOD and RANSEL kits from RANDOX Laboratories Ltd. (Crumlin, UK). Catalase activity was measured according to the procedure described by Bartosz [20]. Serum free thiol content (THIOL) was determined with Ellman's reagent (Sigma Chemical Co., USA) [21] and albumin level-adjusted, with the aid of bromocresol green method (Stamar, Dabrowa Gornicza, Poland). For the purpose of correlation analysis the data on adipocyte fatty acid-binding protein (A-FABP) were retrieved from a previous study [10]. Triglycerides, total and HDL-cholesterol were measured with enzymatic-colorimetric methods (Konelab 60i analyzer; Thermo Electron Oy, Vantaa, Finland) using Triglycérides Enzymatique PAP 150 and Cholestérol Enzymatique PAP from BioMérieux (Marcy-l'Etoile, France) and HDL-C direct (Randox Laboratories Ltd., Crumlin, UK). LDL-C was calculated using Friedewald formula [22]. Glucose was measured by enzymatic amperometry using Ebio glucose analyzer with glucose oxidase containing electrodes (Eppendorf AG, Hamburg, Germany). Insulin was measured with a chemiluminescence immunoassay (Immulite 2000 analyzer; DPC, Los Angeles, USA).

Statistical Analysis

Data distribution and equality of variances were tested using Kolmogorov-Smirnov and Levene tests; log-transformation was done when appropriate. If not otherwise stated, the data is presented as means with SD and analyzed using ANOVA, t-test for independent samples or Welch-test. Pearson (r) test was used for correlation analysis with outliers detected (Tukey method) and removed prior to analysis (plots checked for linearity). Analysis of covariance (ANCOVA) was used for adjusting for potential confounders (BMI, age, gender, Tanner stage, and HDL-C). Multiple regression was used to quantify the strength of identified associations (stepwise method; residual plots examined for linearity). Frequency analysis was conducted using Fisher or χ^2 tests. All p-values were two-sided and $p \leq 0.05$ was considered significant. All the analyses were performed using MedCalc® software, version 11.5.1.0 (Mariakerke, Belgium).

Results

Arylesterase activity of PON-1 was diminished in association with overweight/obesity, central obesity, high blood pressure, and hyperinsulinemia. All these association remained significant following gender-, age-, and Tanner stage-adjustment; however, the associations with high blood pressure and hyperinsulinemia did not persist after the adjustment for HDL-C and/or BMI. The analysis conducted separately for girls and boys revealed that the drop in enzyme activity was statistically significant only in girls, in whom only the association with general and central obesity remained significant following HDL-C and/or BMI-adjustment (Table 2).

In univariate analysis, PON1 inversely correlated with BMI, A-FABP, and CRP in the whole cohort. However, only the correlation with CRP persisted after the adjustment for all potential confounders. In girls, PON1 correlated with BMI,

Table 2. Paraoxonase-1 activity in children and adolescents stratified by gender and their metabolic profile

Tabela 2. Aktywność paraoksonazy-1 u dzieci i nastolatków z uwzględnieniem płci i profilu metabolicznego

Parameter (Wskaźnik)		Whole cohort (Cała populacja)		Girls (Dziewczęta)		Boys (Chłopcy)	
		mean ± SD, n	P value	mean ± SD, n	P value	mean ± SD, n	P value
Gender (Płeć)	girls boys	146.4 ± 30.9 U, 87 139.6 ± 32.6 U, 69	0.188				
Weight status (Kategoria wagowa)	normal overweight obese	159.2 ± 33 U, 47 134 ± 38.9 U, 27a 136.1 ± 24.9 U, 82a	< 0.0001 (a,g,T,H)	161.1, 23 128.1, 16a 133.2, 48a	0.001 (a,T,H)	157.3, 24 142.5, 11 140, 34	0.098
MetS	no yes	144 ± 34.9 U, 106 139.8 ± 24.7 U, 50	0.390	139.5, 61 139.9, 26	0.967	150, 45 139.7, 24	0.190
Central obesity (Otyłość brzuszna)	no yes	157.6 ± 32.8 U, 52 135.2 ± 28.9 U, 104	< 0.0001 (a,g,T,H,B)	161.6, 24 131.3, 63	< 0.001 (a,T,H,B)	154.1, 28 141.2, 41	0.088
High TG (Wysokie TG)	no yes	140.3 ± 33.5 U, 91 145.9 ± 29.6 U, 65	0.287	137.2, 52 143.4, 35	0.386	144.2, 38 148.8, 30	0.549
Low HDL-C (Niski HDL-C)	no yes	143.3 ± 32.6 U, 144 135.5 ± 22.4 U, 12	0.419	137.7, 73 128.8, 7	0.484	145.5, 55 144.8, 5	0.967
Hyperglycemia (Hiperglikemia)	no yes	141.8 ± 34.6 U, 110 144.6 ± 24.7 U, 46	0.481	134.9, 58 142.4, 22	0.354	145.9, 38 146.6, 24	0.936
High BP (Wysokie ciśnienie)	no yes	146.8 ± 31.2 U, 107 133.7 ± 32.1 U, 49	0.017 (a,g,T)	145, 57 129.5, 30	0.034 (a,T)	148.7, 50 140.4, 19	0.318
Insulin resistance (Insulinooporność)	no yes	146.1 ± 37.1 U, 80 139.1 ± 25.1 U, 76	0.168	138, 37 136.5, 44	0.842	149, 31 142.5, 32	0.430
Hyperinsulinemia (Hiperinsulinizm)	no yes	148.4 ± 36.1 U, 84 135.9 ± 24.8 U, 72	0.012 (a,g,T)	146.9, 48 130.7, 39	0.020 (a,T,H)	150.4, 36 142.1, 33	0.270

significant following adjustment to [a] age, [g] gender, [T] Tanner stage, [H] HDL-cholesterol, and/or [B] BMI.
MetS – metabolic syndrome; TG – triglycerides; HDL-C – high density lipoprotein cholesterol; BP – blood pressure.

znamienne po uwzględnieniu [a] wieku, [g] płci, [T] dojrzałości płciowej (skala Tannera), [H] cholesterolu HDL, i/lub [B] BMI.
SD – odchylenie standardowe; MetS – zespół metaboliczny; TG – triglicerydy; HDL-C – cholesterol HDL; BP – ciśnienie krwi.

A-FABP, SOD1, CAT, GPx1, THIOL, and CRP, of which SOD1 and GPx1 remained significant also in the adjusted models. In boys, PON1 correlated with A-FABP, CRP, and HOMA with CRP and HOMA remaining significant after the adjustment for potential confounders (Table 3).

Of the variables associated with PON1 activity independently from potential confounders only central obesity was retained in multivariate analysis model, explaining 11% in PON1 variability in the whole cohort and 17% variability in girls. In boys, CRP was independently associated with PON1 activity explaining 12% in its variability (Table 3).

There were no significant differences between individuals with phenotypes A and B of PON1 concerning gender distribution (p = 0.785), weight status (p = 0.770) and the frequency of central obesity (p = 0.991), metabolic syndrome (p = 0.393), high blood pressure (p = 0.990), hyperinsulinemia (p = 0.920) and insulin resistance (p = 0.465), nor the elevation of any evaluated metabolic or biochemical parameter.

Discussion

The studies conducted in animals consistently demonstrate antiatherogenic properties of PON1 but its association with human cardiovascular disease remains under debate [1]. Data on PON1 and risk factors for cardiovascular disease in juvenile

Table 3 Significant associations between PON1 activity and metabolic, inflammatory, oxidative stress indices

Tabela 3. Istotne związki między aktywnością PON1 a wskaźnikami metabolicznymi, zapalenia i stresu oksydacyjnego

	Whole cohort (Cała populacja) (r)	Girls (Dziewczęta) (r)	Boys (Chłopcy) (r)
BMI	-0.28, $p < 0.001^{a,g,T,H}$	-0.32, $p = 0.002^{a,T,H}$	NS
A-FABP	-0.31, $p = 0.0001^{a,g,T,H}$	-0.31, $p = 0.005^{a,T,H}$	-0.28, $p = 0.026^{a,T,H}$
CRP	-0.29, $p < 0.001^{a,g,T,H,B}$	-0.26, $p = 0.015^{a,T,H}$	-0.33, $p = 0.006^{a,T,H,B}$
SOD1	NS	0.24, $p = 0.032^{a,T,H,B}$	NS
CAT	NS	-0.23, $p = 0.039$	NS
GPx1	-0.17, $p = 0.046^{a,T,H}$	-0.29, $p = 0.009^{a,T,H,B}$	NS
THIOL	NS	0.25, $p = 0.021^{a,T,H}$	NS
HOMA	NS	NS	-0.31, $p = 0.018^{a,T,H,B}$
Multiple regression (stepwise)	entered: CO, BMI, CRP adjusted to: gender, age, Tanner, HDL-C included: CO (b = –22.6, p < 0.0001) $R^2 = 0.11$, F = 18.7 p < 0.001; const. = 157.8	entered: CO, BMI, SOD1, GPx1 adjusted to: age, Tanner, HDL-C included: CO (b = –29.9, p = 0.0001) $R^2 = 0.18$, F = 16, p < 0.001;const. = 162.1	entered: CRP, HOMA adjusted to: age, Tanner, HDL-C, BMI included: CRP (b = –22.2, p = 0.007) $R^2 = 0.12$, F = 7.9 p = 0.007; const. = 144.5

r – Pearson correlation coefficient; significant following adjustment to [a] age, [g] gender, [T] Tanner stage, [H] HDL-cholesterol, and/or [B] BMI in analysis of covariance (ANCOVA). A-FABP – adipocyte fatty acid-binding protein; CAT – catalase; SOD1 – copper-zinc superoxide dismutase; GPx1 – glutathione peroxidase-1; HOMA – homeostasis model of assessment; CO – central obesity (dichotomous); R^2 – coefficient of determination, b – standardized regression coefficient.

population are confined to one study which linked the drop in PON1 activity with obesity [5]. The authors confirmed this association, however in the context of central rather than general obesity. Taking into account anti-inflammatory and antioxidant properties of PON1 [1], decrease in the enzyme's activity might exacerbate the inflammation and oxidative stress which accompany juvenile obesity.

Concerning the other components of the metabolic syndrome, the authors also found PON1 to be decreased in high blood pressure and hyperinsulinemia, which, however, occurred to be mediated by the differences in BMI. Corroborating the findings from adults [23], PON1 arylesterase activity in this cohort inversely but weakly correlated with BMI.

Contrary to the findings in adults [23], the authors observed no correlation between PON1 and HDL-C. Present results might be affected by a low variability of HDL-cholesterol levels in the patients, in whom dyslipidemia manifested mainly by high triglycerides. However, authors' observations are consistent with findings from general juvenile population [24] as well as from overweight/obese children [5]. Ferretti et al. [2] demonstrated obesity-related decreases in PON1 in adults to be independent from co-occurring reduction in HDL particles. Conversely, Birjmohun et al. [4] showed them to be abolished following the adjustment to HDL-related parameters. These authors have suggested PON1 association with coronary artery disease to be confounded by HDL-cholesterol. However, HDL-C adjustment did not affect the associations observed in this cohort. Together with the observation on diminished PON1 activity despite normal HDL-C, the above stated fact might imply that PON1 reduction in juvenile population is potentially relevant for atherosclerosis development independently from HDL.

In line with stronger antioxidant capacity attributed to A phenotype of PON1 [1], a weak association between R allele (B phenotype) and a higher risk for coronary heart disease has been shown in a meta-analysis [25]. Accordingly, the authors sought differences in the frequency of obesity-related metabolic disorders between PON1 phenotypes but found none. They did not differ when it comes to the levels of metabolic and biochemical indices as well.

Koncos at al. [5] demonstrated PON1 association with adipocytokines – a positive one with adiponectin and a negative one with leptin. The authors have also found PON1 to negatively correlate with another adipose tissue-derived protein – A-FABP, the levels of which increase along with obesity [26]. However, contrary to leptin and adiponectin, A-FABP correlation with PON1 lost significance when BMI-adjusted.

Low-grade inflammation is believed to underlie obesity as well as related metabolic abnormalities. Elevated CRP levels are found in obese, insulin-resistant, dyslipidemic, and/or diabetic individuals and are shown to predict cardiovascular risk independently from LDL-C and HDL-C levels as well as other components of the metabolic syndrome [27]. Authors' observation on inverse relation between PON1 and CRP, independent from potential confounders, is consistent with findings demonstrating CRP and inflammatory cytokines to affect serum PON1 activity and directly reduce the number of PON1 transcripts in the liver [28] and supports the results from adults [4,23].

Sumegova et al. [24] reported higher PON1 arylesterase activity in girls than boys in a population-based study. There was a similar tendency in this cohort, which, however, did not reach statistical significance. Nevertheless, the authors found differences between genders concerning PON1 associations. PON1 seems to be more tightly associated with oxidative stress-related obesity in girls whereas in boys – with inflammation. Exclusively in girls, PON1 activity corresponded with erythrocyte antioxidant enzymes and serum free thiol content. The trend of the association differed, being positive for SOD1 and free thiol content and negative for GPx1 and catalase. Accordingly, SOD1 and thiol content were decreased and GPx1 and catalase increased in association with central obesity in this cohort (unpublished observations). Correlations with GPx1 and SOD1 were BMI-independent but lost their significance only when central obesity was accounted for. Inflammation, in turn, mediated an inverse association with HOMA found exclusively in boys. Authors' observations imply that PON1 might be differently regulated in both genders pointing at obesity and related oxidative stress in girls and inflammation in boys as important factors affecting its arylesterase activity.

Summarizing, the authors showed PON1 to decrease in association with central rather than general obesity. The factors independently associated with decreases in PON1 were found to be gender-dependent: obesity and obesity-related oxidative stress in girls and inflammation in boys.

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