

Overweight and Obesity in Polycystic Ovary Syndrome: Association with Inflammation, Oxidative Stress and Dyslipidemia

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Abstract:

Objective: Polycystic ovary syndrome (PCOS) is associated with altered lipid profile and increased small, dense LDL particles (sdLDL). Considering that paraoxonase 1 (PON1) is an anti-oxidative enzyme located on high-density lipoprotein (HDL) particles, the aim of this study was to investigate the connection between oxidative stress (OS) and PON1 activity with lipoprotein subclasses in PCOS depending on obesity. **Methods:** In 115 PCOS patients lipoprotein subclasses distributions were determined by gradient gel electrophoresis. OS status was assessed by total oxidative status (TOS), advanced oxidation protein products (AOPP), malondialdehyde (MDA), prooxidant-antioxidant balance (PAB), total antioxidant status (TAS) and superoxide dismutase (SOD) and PON1 activity. **Results:** Overweight/obese PCOS patients (n=55) had increased OS compared to normal weight patients (n=60). In addition, overweight/obese group had lower HDL size and higher proportion of HDL 3a subclasses (P<0.05). PAB was in negative correlation with HDL 2a (P<0.001), whereas MDA and SOD correlated positively with HDL 3 subclasses (P<0.05). Serum PON1 activity was positively associated with proportions of PON1 activity on HDL 2b (P<0.05) and 2a (P<0.01), but negatively with the proportion on HDL 3 particles (P<0.01). LDL B phenotype patients had increased TAS, SOD and PON1 activity on HDL 2b, but decreased PON1 activity on HDL 3 subclasses. **Conclusion:** OS is associated with altered lipoprotein subclasses distribution in PCOS patients. Obesity in PCOS affects the profile of HDL subclasses, reflected through the reduced proportion of PON1 activity on HDL 3 subclasses in the presence of sdLDL particles.

Keywords: Obesity, PCOS, inflammation, oxidative stress, dyslipidemia

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women in the reproductive period [1]. Because of its heterogeneity, elusiveness regarding etiology and diagnostic difficulties, PCOS is a complex endocrine and metabolic condition. The key risk factors which affect cardiovascular disease (CVD) risk in PCOS with complex mechanisms are dyslipidemia, increased oxidative stress (OS) [2] and chronic low-grade inflammation [3].

Recent studies have shown that women with PCOS, especially obese patients, have a significantly altered lipid profile characterized by qualitative changes of low-density lipoproteins (LDL), in terms of increased small, dense LDL particles (sdLDL), hypertriglyceridemia and reduced HDL-C concentrations [4]. This so-called “*atherogenic lipoprotein phenotype*” (ALP) represents a form of dyslipidemia that contributes to greater CVD risk [5]. Namely, sdLDL comprises LDL particles smaller than 25.5 nm, which are considered as the most atherogenic LDL subfractions. In addition, increased levels of oxidized LDL (oxLDL) were confirmed in women with PCOS, suggesting that lipid metabolism in PCOS is associated with OS [6].

OS is defined as an imbalance between excessive formation of reactive oxygen species (ROS) and reduced antioxidant defence capacity. The pathophysiological mechanism of OS contribution in PCOS development remains vague, but numerous studies have found that PCOS is associated with OS [7] [8]. We recently assessed OS biomarkers and proposed the evaluation of OXY-score as a comprehensive measure of OS status in women with PCOS [9].

High-density lipoprotein (HDL) constitutes a heterogeneous group of particles [10] and previous studies suggested a relationship between altered HDL subclasses distribution and CVD risk [11]. Current research of anti-atherogenic roles of HDL particles is focused on evaluation of their functionality in addition to HDL subclasses distribution. However, data on HDL particles distribution and functionality in PCOS are scarce.

The antioxidative/anti-inflammatory role of HDL particles depends on the presence of antioxidant enzymes. Paraoxonase 1 (PON1) is a circulating esterase and lactonase associated with apolipoprotein A1 on HDL particles [12]. Antioxidant activity of PON1 is achieved by hydrolysis of lipid peroxides in oxidized lipoproteins' [13] [14]. OS contributes to reducing PON1 activity by changing the redox status of free sulfhydryl groups of the protein molecule of this enzyme [15]. It is also clearly established that PON1 preferentially associates with small HDL 3 particles and then shifts to larger HDL 2 subclasses during the course of HDL maturation in the reverse cholesterol transport process [16]. However, different pathological

conditions may affect HDL maturation and, consequently, the distribution of PON1 between HDL 2 and HDL 3 particles [17].

Taking all these facts together, we hypothesized that dyslipidemia, oxidative stress, and inflammation could be related with pro-atherogenic changes in lipoprotein subclasses profiles in women with PCOS. Therefore, the aim of this study was to analyze the association of OS status with HDL and LDL particle size and subclasses, as well as with PON1 activity distribution on HDL subclasses in women with PCOS, especially regarding their obesity status.

Material and methods

Subjects and ethics

In total, 115 PCOS patients, 60 normal weight women (mean BMI: $21.24 \pm 1.98 \text{ kg/m}^2$) and 55 overweight or obese women (mean BMI: $32.26 \pm 5.4 \text{ kg/m}^2$), aged between 29 and 39 years, were included in this study. The patients were recruited at the Clinic of Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Belgrade. PCOS was defined based on the revised Rotterdam Consensus Criteria (2003) [18]. All patients were recruited during the early follicular phase of the menstrual cycle, or at any time if they had severe oligomenorrhea or amenorrhea.

Written informed consent was voluntarily obtained from all subjects. The whole study was planned and conducted respecting the principles of the Declaration of Helsinki (Edinburgh, 2000). The study protocol was evaluated and approved by Ethics Committees of the Faculty of Medicine and Faculty of Pharmacy, University of Belgrade.

Anthropometric measurements

Measurements of body weight and height, waist and hip circumferences (WC and HC) were carried out in the morning in a fasting state and BMI and waist-to-hip ratio (WHR) were calculated.

Biochemical analyses

Blood samples were collected in the morning after a 12 h fasting period in the follicular phase of the cycle (on the 2nd or the 3rd day). Plasma and serum were separated and stored until analyses.

Fasting serum glucose, total cholesterol (TC), HDL-C, triglycerides (TG) and C-reactive protein (CRP) concentrations were determined using commercially available enzymatic and turbidimetric tests on Dimension RxL Max (Siemens Healthcare GmbH,

Germany) analyser. The concentration of LDL-C was calculated by the Friedewald formula [19].

Total oxidative status (TOS), advanced oxidation protein products (AOPPs), malondialdehyde (MDA), prooxidant-antioxidant balance (PAB), total antioxidative status (TAS), superoxide dismutase (SOD) and paraoxonase 1 (PON1) were measured on ILab 300⁺ (Instrumentation Laboratory, Milan, Italy). For redox status parameters, the intra-assay and inter-assay coefficients of variance for all assays were below 4.3% and 8.2%, respectively.

Plasma HDL and LDL particles were separated using a non-denaturing 3-31% polyacrylamide gradient gel electrophoresis method [20]. The HDL and LDL particle size determination and subclasses analyses were described in detail and published elsewhere [21]. In brief, the sizes of LDL and HDL particles were determined based on migration distance of the major peaks in densitometric profile, from calibration curve made from high molecular weight protein standards, carboxylated polystyrene microsphere beads and human plasma with two LDL subclasses of known diameters. Relative proportions of LDL and HDL subclasses were determined by analysing areas under the peaks that correspond to the known ranges of particle diameters: LDL I (27.2-28.5 nm), LDL II (25.5-27.2 nm), LDL III (24.2-25.5 nm), LDL IV (22.0-24.2 nm), HDL 2b (9.7-12.0 nm), HDL 2a (8.8-9.7 nm), HDL 3a (8.2-8.8 nm), HDL 3b (7.8-8.2 nm) and HDL 3c (7.2-7.8 nm). Based on LDL size, the patients were further classified as the carriers of LDL phenotype A (LDL diameter >25.5 nm) or LDL phenotype B, characterized by a predominance of sdLDL particles (LDL diameter ≤25.5 nm) [21]. Paraoxonase 1 (PON1) activity distribution on HDL subclasses was determined by the zymogram method according to Gugliucci et al. [16]. The areas under the peaks of densitometric scans of the sample represent the relative proportion of PON1 activity in particular HDL subclass, as previously described [17].

Statistical analysis

The data are shown as mean ± standard deviation for normally distributed variables or as median and interquartile range for non-normally distributed parameters. Differences among continuous variables were evaluated using Student's t test for parametric data or Mann-Whitney U test for non-parametric data. Since age differences between overweight/obese and normal weight PCOS patients were significant, ANCOVA and Quade's tests (non-parametric ANCOVA) were applied for further analysing of parametric and non-parametric data, respectively, with age as a covariate. Spearman's correlation analysis was performed to assess the association between lipoprotein subclasses, OS parameters, PON1 activity on HDL

subclasses and risk scores. To determine the Dyslipidemia score we standardised all the variables by calculating Z scores for LDL-C, TG and HDL-C using mean and standard deviation calculated from healthy population data from our laboratory. Thereafter, the Dyslipidemia score was calculated according to the equation: average of the standardised variables (LDL-C+TG)/2 minus standardised HDL-C. The OXY-score was calculated by subtracting the Protective score from the Damage score using the equation given by Veglia et al. [22]. The Protective score was calculated as an average of standardised antioxidant variables (TAS, SOD and PON1 activity) while the Damage score was calculated as an average of standardised prooxidant factors (AOPP, TOS, PAB and MDA). The Inflammation score was determined in both groups after standardisation of CRP as an acute phase protein. Dyslipidemia-Oxy-Inflammation (DOI) score was calculated as the sum of the Dyslipidemia score, OXY-score, and the Inflammation score. All variables with non-normal distributions were log-transformed before computation. To determine the predictors of LDL phenotype B in PCOS, univariate logistic regression analysis was performed. Dependent variable was LDL phenotype, risk group included PCOS patients with LDL B phenotype, while the patients with LDL A phenotype formed the reference group. As independent variables, age, BMI and those that were significantly different between the patients with LDL phenotypes A and B (TG, TAS, SOD and PON1 activity on HDL 2b, HDL 3b and HDL 3c subclasses) were tested. Multivariate logistic regression analysis (backward selection) was applied to predictors which in univariate analysis reached P value equal or less than 0.100 (TG, TAS, SOD and PON activity on HDL 2b, HDL 3b and HDL 3c subclasses) in order to determine the best model consisted of independent predictors of LDL phenotype B. Statistical analysis was performed using the Statistical Package for the Social Sciences (version 22, SPSS Inc. Chicago, USA). P value <0.05 was considered statistically significant for all analyses.

Results

The demographic, biochemical and lipoprotein subclasses distribution data in normal weight and overweight or obese PCOS patients are presented in Table 1. As expected, normal weight PCOS patients had significantly lower BMI, WC and WHR compared to overweight or obese PCOS patients. Overweight/obese PCOS patients were older than their normal weight counterparts. Fasting glucose concentration was similar in both groups, but the levels of CRP were significantly higher in overweight or obese PCOS patients. The levels of TC, TG and LDL-C were significantly higher, while HDL-C was significantly lower in overweight or obese (P<0.001) PCOS patients and the differences remained significant after adjustment for

age. No significant differences were found in LDL particle size and subclasses distribution between the two groups of PCOS patients. On the other hand, overweight or obese PCOS women had significantly lower HDL particle diameters ($P<0.05$) and higher proportion of HDL 3a subclasses compared to normal weight PCOS patients ($P<0.05$), even after adjustment for age.

The characteristics of OS status in both PCOS groups are shown in Figure 1. Significantly higher levels of prooxidant parameters (TOS and AOPP) were found in overweight or obese PCOS patients compared to normal weight PCOS patients. The observed differences between the two PCOS groups persisted after adjustment for age. We did not find significant differences in PON1 activity (Figure 1) and PON1 distribution on HDL subclasses (data not shown) between normal weight and overweight or obese PCOS groups.

Table 2 shows the results of Spearman's correlation analysis between lipoprotein data and parameters of OS status among all PCOS patients (overweight/obese and normal weight women). We found that the smaller LDL particle diameters were in correlation with higher levels of AOPP. Relative proportion of LDL I subclasses was positively associated with TAS and negatively with PAB. The proportion of small LDL IV subclasses was in positive correlation with PAB and in negative correlation with TAS. The level of PAB was in positive correlation with HDL 2b, while in negative correlation with the proportion of HDL 2a subclasses. A significant positive correlation was found between SOD activity and proportion of HDL 3b subclasses, as well as between concentration of MDA and proportion of HDL 3a subclasses.

Serum PON1 activity was positively associated with relative proportion of PON1 activity on HDL 2b ($\rho=0.204$, $P<0.05$) and HDL 2a ($\rho=0.335$, $P<0.01$), and negatively with the proportion on HDL 3a ($\rho=-0.312$, $P<0.01$) and HDL 3b ($\rho=-0.332$, $P<0.01$) subclasses. In addition, PON1 activity on HDL 3b particles positively correlated with TAS ($\rho=0.226$, $P<0.05$) and TOS ($\rho = 0.207$, $P<0.05$), and negatively with PAB ($\rho=-0.296$, $P<0.01$). PON1 activity on HDL 3c particles was in positive correlation with TOS ($\rho = 0.207$, $P<0.05$).

To gain further insight in the interactive role of OS, dyslipidemia and inflammation, risk scores were calculated. As shown in Figure 2, all the scores, except Protective score, were higher in overweight or obese PCOS patients. Smaller LDL size was associated with higher Dyslipidemia ($\rho=-0.223$, $P<0.05$), Damage ($\rho = -0.241$, $P<0.05$) and OXY ($\rho=-0.213$, $P<0.05$) scores. An inverse association was found between HDL size and Dyslipidemia score ($\rho=-0.201$, $P<0.05$), as well as between PON1 activity on HDL 3 subclasses and Protective score ($\rho=-0.228$, $P<0.05$).

Next, we assessed the impact of LDL phenotype on lipid, OS status and PON1 activity distribution on HDL subclasses in PCOS (Table 3). LDL phenotype B patients showed higher TG concentration, but HDL-C concentrations and HDL size did not differ between the groups. The data also showed significantly higher levels of parameters of antioxidant protection (TAS and SOD) in LDL phenotype B group. PON1 activity on HDL 2b subclasses was significantly higher, whereas PON1 activity on HDL 3b and HDL 3c subclasses was significantly lower in women with LDL phenotype B. Serum PON1 activity was not different between PCOS patients with LDL A and B phenotypes.

The concentration of TG, activity of SOD in the serum and the proportion of PON1 activity on HDL 3b particles were indicated as significant predictors of LDL phenotype B (Table 4). In the multivariate analysis, PON1 activity on HDL 3b particles remained significantly associated with LDL phenotype B (OR = 0.781, 95% CI: 0.614-0.993; $P < 0.048$), the association of SOD activity was of borderline significance (OR = 1.024, 95% CI: 0.999-1.43; $P = 0.059$), while TG lost significance revealed in univariate analysis.

Discussion

Dyslipidemia is the most common metabolic abnormality in PCOS, although the type and the extent of the disorder varies [2], as a result of complex interactions between hyperandrogenism, IR, environmental (nutrition, physical activity) and genetic factors [23] [24]. Previous studies have shown that obese women with PCOS may have reduced LDL particle size, due to the increased sdLDL particles [25]. In the study of Phelan et al. [4], both PCOS and BMI were associated with decreased LDL size and redistribution towards smaller, pro-atherogenic particles. They concluded that, despite the fact that exact mechanisms of changed lipoprotein metabolism in PCOS are not known, hyperandrogenemia and increased central obesity could be causal factors. We assume that excess of androgen early in life of normal weight PCOS patients might be the reason for the absence of a significant difference in LDL particle size and subclasses between our PCOS groups. It is known that many factors, including BMI, ethnic origin, complexity of the syndrome (anovulatory vs. ovulatory) and diet have an impact on LDL-C in PCOS patients. In line with previous studies, the same factors may also influence LDL particles distribution [21]. So far, only few studies dealt with HDL subclasses distribution in PCOS. In the current study, overweight or obese PCOS patients had significantly smaller HDL particle diameters and higher proportions of small HDL 3a subclasses compared to normal weight patients (Table 1). This finding is in agreement with the available data which showed a decrease in the HDL particle size in obese

PCOS patients and other groups with higher CVD risk [26], suggesting that obesity is the major contributor for the observed changes in HDL subclasses profile in PCOS.

The results of previous studies have shown that women with PCOS have elevated markers of OS, as well as that PCOS, independently of obesity, may trigger OS [8]. In our study, TOS, an aggregative index of plasma oxidant state, and AOPP, a marker of oxidant-mediated protein damage and a potent pro-inflammatory mediator, were increased in overweight or obese PCOS patients (Figure 1). This agrees with the findings of Blair et al. which showed significant difference in TOS levels between PCOS patients with respect to BMI [27]. Our previous study showed that PCOS patients had lower PON1 activity compared to the control group [9]. In the current study, however, PON1 activity and PON1 distribution on HDL subclasses did not differ between normal weight and overweight or obese PCOS patients (Figure 1). These results suggest that disease itself affects serum PON1 activity and distribution on HDL subclasses, regardless of BMI, which should be confirmed in the future studies in order to improve our understanding of PON1 status in PCOS.

The results of the presented study demonstrated significant interplay between markers of OS status and lipoprotein subclasses distributions in PCOS. The markers of enhanced OS (AOPP, PAB) were associated with sdLDL particles, which may indicate higher potential for their oxidative modifications. The levels of oxLDL are increased in PCOS patients and represent a reliable predictor of CVD, as has been confirmed in the study of Macut et al. [6]. The observed associations between the levels of PAB and relative proportions HDL 2 particles suggests that OS might also play the role in alterations of HDL metabolism in PCOS. In fact, OS is able to inhibit lecithin-cholesterol acyltransferase (LCAT) activity and consequently to affect the maturation of HDL particles, leading to the accumulation of small HDL 3 subclasses [17]. Furthermore, free radicals can also modify HDL particles [28]. In support of this hypothesis, our data showed that higher proportions of HDL 3a subclasses were associated with increased MDA concentrations, which is consistent with previous results [21]. Yet, the question that still remains disputable is which HDL subclass provides the strongest cardiovascular protection. It has been suggested that larger HDL 2 particles have higher atheroprotective potential, because their levels are reduced in patients with CVD or higher in premenopausal women [29]. However, *in vitro* studies have demonstrated that smaller HDL 3 particles are more effective cholesterol acceptors and also have superior antioxidant properties than larger HDL 2 particles [30]. Another controversy is centred around the exact localization of PON1 on HDL subclasses. Previous studies have determined that PON1 activity is most commonly found on smaller HDL 3 particles [30]. Recent HDL

proteomic studies have confirmed these findings [31], supporting the concept that smaller HDL 3 particles have greater antioxidative potential. Recently, a hypothesis has been raised that the localization of PON1 changes during maturation of HDL particles. Hence, the inverse association between serum PON1 activity with the proportions of PON1 activity on HDL 3 subclasses observed in this current study can be explained by the rapid transfer from smaller HDL 3 to larger HDL 2 particles [29]. Moreover, our analysis showed that the proportion of PON1 activity on HDL 3 subclasses was associated with OS biomarkers. This finding suggests that exacerbated OS in PCOS may affect HDL maturation that yields to the accumulation of PON1 on smaller HDL 3 subclasses. On the other hand, there is also a possibility that the observed associations reflect superior antioxidative properties of small HDL 3 particles in the pro-oxidative conditions, such as PCOS [9]. The observed inverse association between PON 1 activity on HDL 3 particles and Protective score, representing general antioxidative potential of plasma, may support this assumption.

Further analysis showed increased TAS and SOD activity in PCOS patients with LDL B phenotype, whereas the indices of enhanced OS did not differ between the two LDL phenotype groups (Table 3). LDL B phenotype denotes increased prevalence of sdLDL particles, which are more susceptible to oxidative modifications [6]. A finding of increased levels of antioxidants in the patients with LDL phenotype B suggests upregulation of antioxidative defence mechanisms to prevent OS exacerbation in PCOS. Analysis of PON1 distribution on HDL subclasses showed that women with LDL phenotype B had lower PON1 activity on HDL 3 subclasses, but higher activity on HDL 2b particles than the patients with LDL A phenotype. This finding can be a sign of reduced antioxidant capacity of HDL 3 particles in conditions associated with increased CVD risk [29], such is the preponderance of sdLDL particles. A potential relevance of the observed associations was further confirmed in logistic regression analysis (Table 4). As we expected, TG concentration was pointed out as an important predictor of LDL B phenotype, which is consistent with the fact that hypertriglyceridaemia is one of the features of ALP. Nevertheless, higher activity of SOD, as well as reduced proportion of PON1 activity on small HDL 3b particles remained significantly associated with LDL B phenotype in multivariate analysis. It is now well established that atherogenesis starts at young age and continues throughout life, especially in the presence of risk factors such as obesity, dyslipidemia, inflammation and elevated OS, that are commonly seen in PCOS. Therefore, the evaluation of novel cardiometabolic risk factors and the understanding of the kinetics and function of PON1, particularly how it is affected in

various conditions that alter maturation and remodelling of HDL particles, provide additional information to guide preventive measures in order to attenuate atherosclerosis progression.

Strengths and Limitations

The current study represents a comprehensive evaluation of OS status and lipoprotein subclasses distributions and provides novel data on PON1 distribution on HDL subclasses in PCOS. The studies that investigate PON1 distribution on HDL subclasses might provide further insight into mechanisms responsible for HDL dysfunction in various diseases. This may be of particular importance in individuals with increased cardiometabolic risk, such as young women with PCOS. The cross-sectional design of our study represents the limitation because it does not allow us to demonstrate the causality of the observed relationships. Future prospective studies with larger number of patients would be needed to further expand our observations. Also, considering that this is a cross-sectional study and not an interventional study, we analyzed two groups of patients on standard diet that were diagnosed based on Rotterdam criteria including only combination of clinical, hormonal and ultrasound indices. We believe that the impact of diet could be used in future investigations to show how dietary interventions influence inflammation, oxidative stress and dyslipidemia in patients during prolonged period of follow-up or on specific therapeutic regimens.

Conclusion

Our data showed that OS is associated with pro-atherogenic changes in lipoprotein subclasses profiles in patients with PCOS. Obesity in the PCOS favours redistribution of HDL subclasses toward smaller particles with a lower antioxidant potential, particularly in the presence of sdLDL particles. Taking all into account, our data provide additional evidence in favour of advanced lipid and OS testing for future management of CVD risk in PCOS patients.

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Author contributions

All authors listed have contributed sufficiently to the study to be included as authors.

Iva M. Perovic Blagojevic- carrying out the study, analysing the data, interpreting the findings, writing the article

Jelena Z. Vekic- analysing the data, interpreting the findings

Djuro P. Macut- formulating the research question(s), designing the study

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Compliance with ethical standards

Conflict of interest: None

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Table 1 Demographic, biochemical data and lipoprotein subclasses distribution in PCOS subgroups

Parameter	PCOS		P
	Normal weight	Overweight or obese	
N	60	55	
Age, years	25.4 ± 4.9	27.3 ± 5.2	<0.05
BMI, kg/m ²	21.24 ± 1.98	32.26 ± 5.4	<0.001
Waist circumference, cm	73.7 ± 7.2	100.1 ± 14.5	<0.001
Waist-to-hip ratio	0.77 ± 0.06	0.87 ± 0.07	<0.001
Glucose, mmol/L	4.87 ± 1.39	5.02 ± 0.46	0.443
CRP, mg/L #	0.75 (0.50-1.35)	3.50 (1.80-6.10)	<0.001
Total cholesterol, mmol/L	4.49 ± 0.89	5.18 ± 0.93	<0.001
HDL-C, mmol/L	1.71 ± 0.47	1.24 ± 0.32	<0.001
LDL-C, mmol/L	2.41 ± 0.81	3.22 ± 0.83	<0.001
TG, mmol/L #	0.71 (0.62-0.91)	1.32 (0.86-2.19)	<0.001
LDL size, nm	27.00 ± 1.34	26.63 ± 1.28	0.131
LDL I, %	21.2 ± 3.1	21.6 ± 3.3	0.526
LDL II, %	25.2 ± 4.2	25.8 ± 4.4	0.456
LDL III, %	21.1 ± 2.7	20.5 ± 3.0	0.307
LDL IV, %	32.5 ± 5.1	32.1 ± 5.9	0.678
sdLDL, %	53.6 ± 5.5	52.7 ± 6.6	0.388
HDL size, nm	10.62 ± 0.79	10.24 ± 0.91	<0.05
HDL 2b, %	52.8 ± 10.6	50.3 ± 9.3	0.192
HDL 2a, %	20.9 ± 7.6	21.4 ± 4.3	0.705
HDL 3a, %	11.5 ± 3.3	13.1 ± 4.2	<0.05
HDL 3b, %	6.3 ± 3.2	6.5 ± 3.2	0.749
HDL 3c, %	8.5 ± 4.6	8.5 ± 3.6	0.966

Data are presented as mean ± standard deviation and compared by Student's t test.

Data are presented as median (interquartile range) and compared by Mann-Whitney U test.

BMI -Body Mass Index, CRP-C Reactive Protein, HDL-C –High-Density Lipoprotein Cholesterol, LDL-C –Low-Density Lipoprotein Cholesterol, TG -Triglycerides, LDL- Low-density Lipoprotein, sdLDL- small, dense Low-density Lipoprotein, HDL- High-Density Lipoprotein

Table 2 Correlations between lipoprotein subclasses and oxidative stress status in PCOS

Parameter	TOS, μmol/L	AOPP, μmol/L	MDA, μmol/L	PAB, U/L	TAS, μmol/L	SOD, U/L	PON1, U/L
LDL size, nm	-0.058	-0.214*	-0.180	-0.111	0.121	-0.132	-0.023
LDL I, %	0.060	0.062	-0.084	-0.287**	0.235*	-0.048	-0.039
LDL II, %	-0.019	-0.037	-0.073	-0.154	0.158	-0.063	0.030
LDL III, %	-0.068	0.002	-0.072	0.175	-0.090	0.068	0.092
LDL IV, %	0.042	0.012	0.183	0.258**	-0.228*	0.055	-0.001
HDL size, nm	0.011	-0.126	0.080	0.050	0.001	0.033	0.075
HDL 2b, %	-0.058	-0.131	-0.087	0.201*	-0.062	-0.090	0.006
HDL 2a, %	0.047	0.050	-0.160	-0.369***	0.169	-0.160	-0.042
HDL 3a, %	0.045	0.136	0.225*	-0.169	0.077	0.055	-0.089
HDL 3b, %	0.020	0.112	0.023	0.026	-0.139	0.187*	0.001
HDL 3c, %	-0.001	0.082	0.074	-0.006	-0.040	0.166	0.022

Data are presented as Spearman's correlation coefficients: * P<0.05; ** P<0.01; *** P<0.001.

TOS- Total Oxidant Status, AOPP- Advanced Oxidation Protein Products, MDA- Malondialdehyde, PAB- Pro-Oxidant Antioxidant Balance, TAS- Total Antioxidant Status, SOD- Superoxide Dismutase, PON1- Paraoxonase-1, LDL- Low-density Lipoprotein, HDL- High Density Lipoprotein

Table 3 Lipid status, oxidative stress status and PON1 activity distribution on HDL subclasses in PCOS patients according to LDL phenotype

Parameter	LDL phenotype B	LDL phenotype A	P
N	15	100	
BMI, kg/m ²	27.1 ± 6.5	26.5 ± 6.9	0.728
Total cholesterol, mmol/L	5.29 ± 0.91	4.76 ± 0.97	0.057
HDL-C, mmol/L	1.41 ± 0.58	1.49 ± 0.45	0.515
LDL-C, mmol/L	3.12 ± 0.89	2.76 ± 0.91	0.162
TG, mmol/L [#]	1.32 (0.90-2.31)	0.83 (0.66-1.37)	<0.05
HDL size, nm	10.22 ± 0.90	10.47 ± 0.87	0.334
TOS, μmol/L [#]	37.7 (30.0-47.4)	37.7 (30.0-48.7)	0.801
AOPP, μmol/L [#]	38.9 (27.4-74.3)	30.7 (23.2-48.8)	0.146
MDA, μmol/L [#]	4.0 (3.3-5.9)	3.5 (2.7-4.5)	0.209
PAB, U/L [#]	102.5 (66.0-138.51)	79.0 (48.0-105.5)	0.207
TAS, μmol/L [#]	1085.0 (902.0-1244.5)	935.0 (716.5-1077.5)	<0.05
SOD, U/L [#]	138.5 (114.5-158.5)	118.0 (105.0-139.5)	<0.05
PON1, U/L [#]	287.5 (212.5-600.5)	230.0 (163.5-479.0)	0.177
PON1 HDL 2b, %	56.2 ± 11.4	50.9 ± 2.4	<0.001
PON1 HDL 2a, %	24.9 ± 4.3	24.0 ± 5.9	0.565
PON1 HDL 3a, %	10.6 ± 2.9	12.0 ± 5.4	0.357
PON1 HDL 3b, %	4.6 ± 2.8	6.6 ± 3.2	<0.05
PON1 HDL 3c, %	3.7 ± 2.5	6.5 ± 5.2	<0.01

[#] Data are presented as median (interquartile range) and compared by Mann-Whitney U test.

BMI- Body Mass Index, HDL-C- High Density Lipoprotein Cholesterol, LDL-C- Low-density Lipoprotein Cholesterol, TG- Triglycerides, HDL- High Density Lipoprotein, TOS- Total Oxidant Status, AOPP- Advanced Oxidation Protein Products, MDA- Malondialdehyde, PAB- Pro-Oxidant Antioxidant Balance, TAS- Total Antioxidant Status, SOD- Superoxide Dismutase, PON1- Paraoxonase-1

Table 4 Univariate logistic regression analysis of LDL phenotype B predictors in PCOS

Parameter	OR (95% CI)	P
Age, years	1.067 (0.959-1.188)	0.234
BMI, kg/m ²	1.014 (0.937-1.099)	0.725
TG, mmol/L	2.152 (1.144-4.046)	<0.05
TAS, μmol/L	0.998 (0.997-1.000)	0.085
SOD, U/L	1.024 (1.004-1.044)	<0.05
PON1 HDL 2b, %	1.051 (0.992-1.114)	0.092
PON1 HDL 3b,%	0.795 (0.646-0.975)	<0.05
PON1 HDL 3c, %	0.852 (0.723-1.003)	0.055

BMI- Body Mass Index, TG- Triglycerides, TAS- Total Antioxidant Status, SOD- Superoxide Dismutase, PON1- Paraoxonase-1, HDL- High Density Lipoprotein

Figure legends:

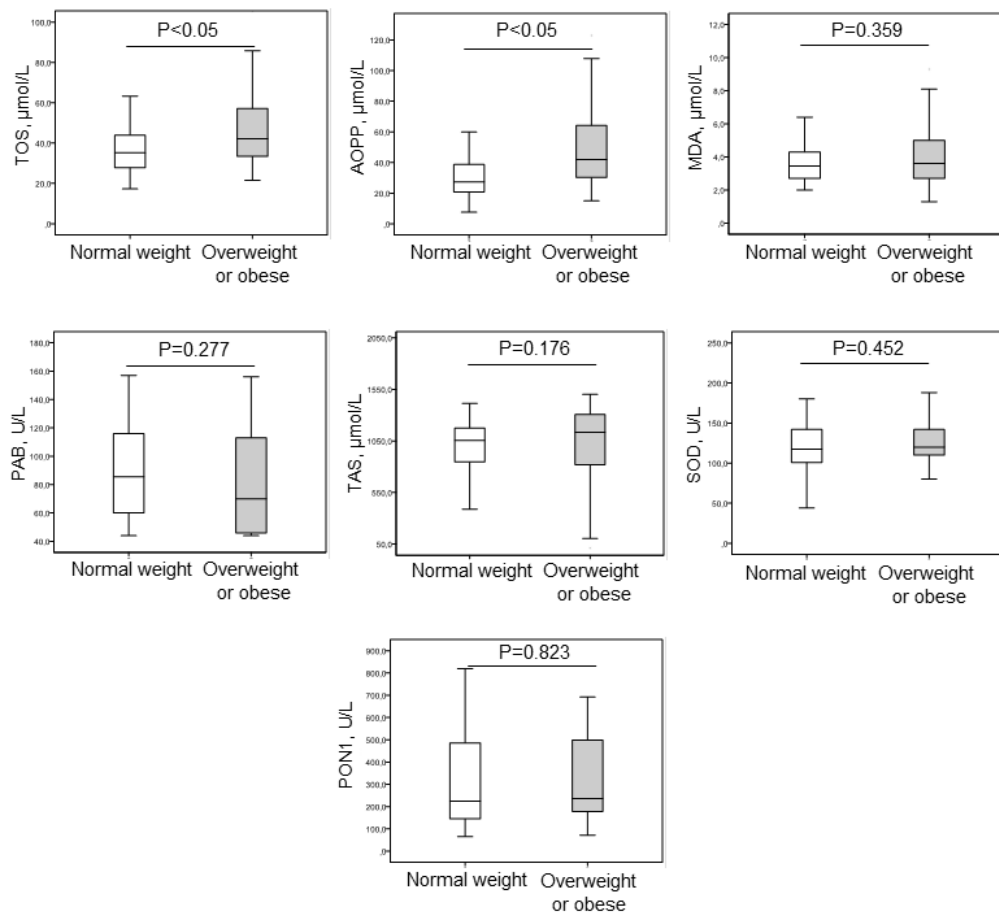


Fig. 1 Oxidative stress status in normal weight and overweight or obese PCOS patients. The box's horizontal edges (lower and upper) indicate the 25th and 75th percentiles, respectively and the horizontal line through the box indicates the median value. Horizontal lines at the lower and upper whiskers indicate minimum and maximum value, respectively.

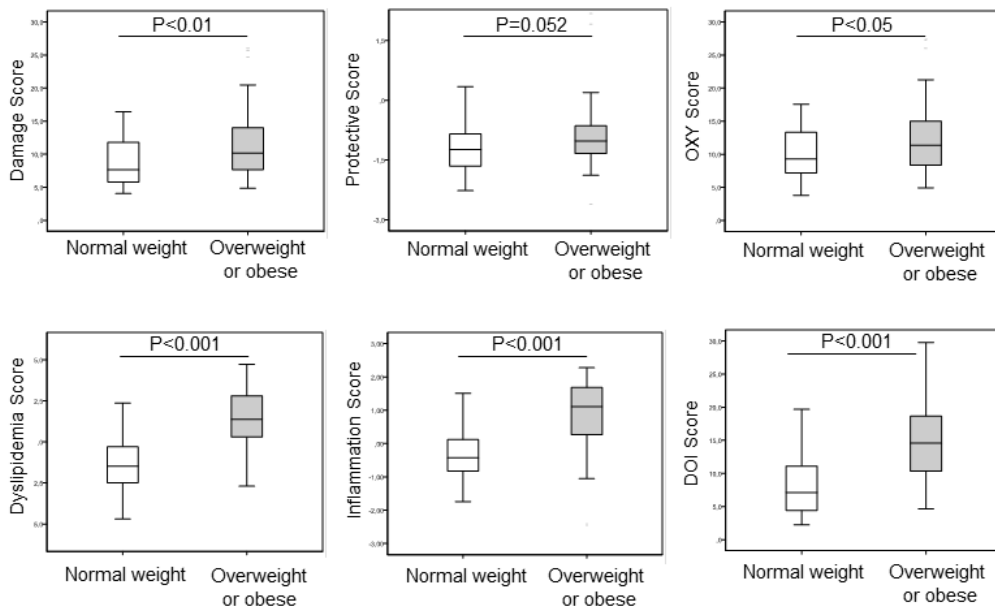


Fig. 2 The scores of oxidative stress, dyslipidemia and inflammation in normal weight and overweight or obese PCOS patients. The box's horizontal edges (lower and upper) indicate the 25th and 75th percentiles, respectively and the horizontal line through the box indicates the median value. Horizontal lines at the lower and upper whiskers indicate minimum and maximum value, respectively.