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IMPACT OF REDOX IMBALANCE AND INFLAMMATION ON ACTIVITY OF PARAOXONASE 1 AND ITS DISTRIBUTION IN HIGH DENSITY LIPOPROTEIN IN POLYCYSTIC OVARY SYNDROME

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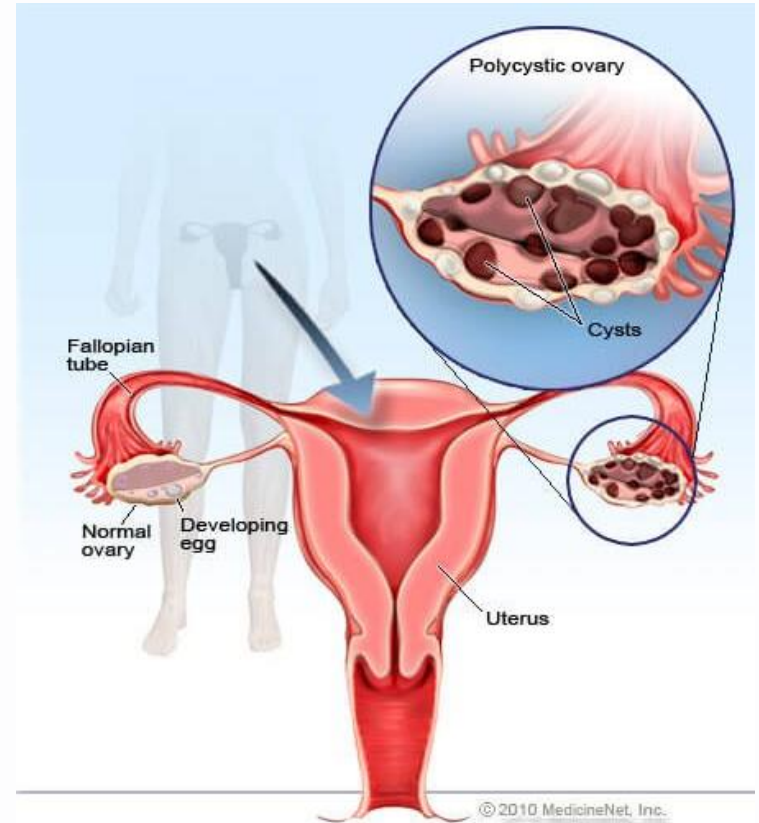
Introduction

PCOS (Polycystic Ovary Syndrome)

The most common endocrine disorder among women of reproductive age

Complex endocrine condition because of:

- ★ heterogeneity,
- ★ inconsistency regarding etiology,
- ★ difficulties in diagnosing



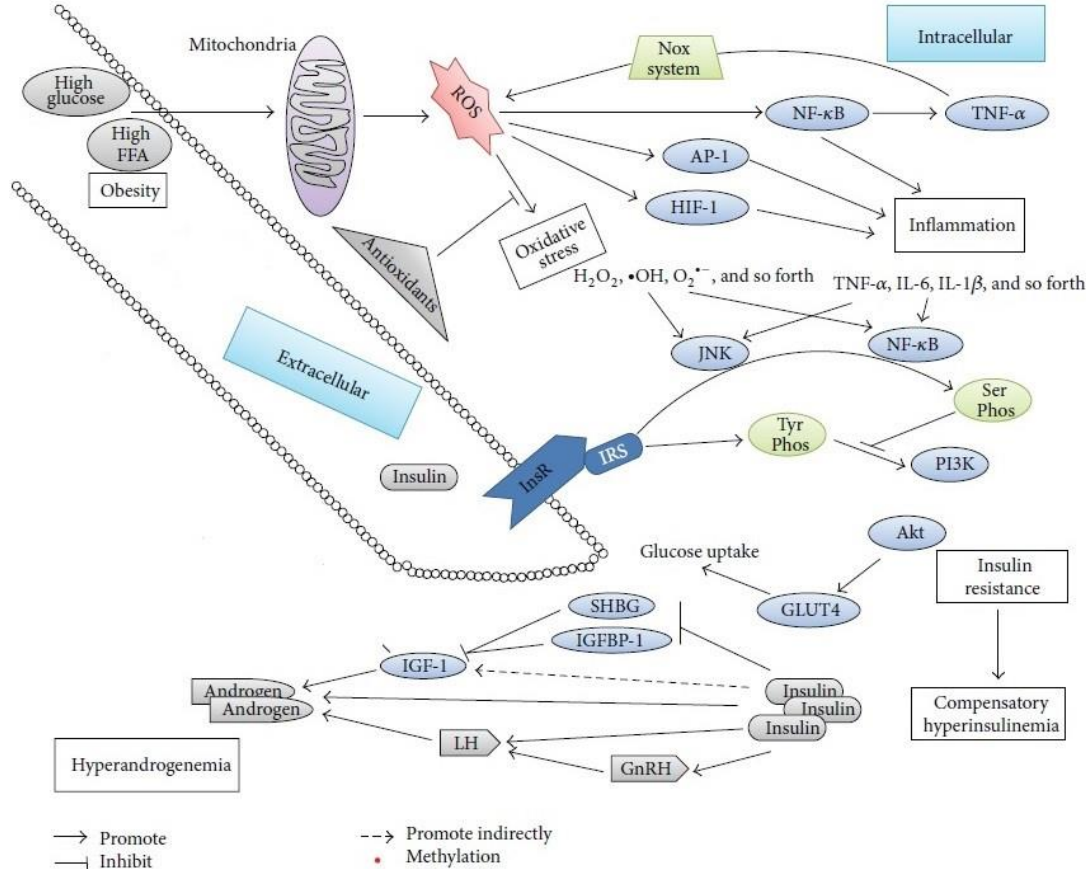
PCOS Diagnostic Criteria

Definition/Year	Diagnostic Criteria
NIH/1990	Requires the simultaneous presence of: <ol style="list-style-type: none">1. Hyperandrogenism (clinical and/or biochemical)2. Ovarian dysfunction
Rotterdam (ESHRE/ASRM) /2003	Requires the presence of at least two criteria: <ol style="list-style-type: none">1. Hyperandrogenism (clinical and/or biochemical)2. Ovulatory dysfunction3. Polycystic ovarian morphology verified by ultrasound
AES/2006	Requires the presence of hyperandrogenism (clinical and/or biochemical) and either: <ol style="list-style-type: none">1. Ovulatory dysfunction2. Polycystic ovarian morphology verified by ultrasound
AES-PCOSS /2009	Requires the simultaneous presence of: <ol style="list-style-type: none">1. Hyperandrogenism (clinical and/or biochemical)2. Ovarian dysfunction (ovulatory dysfunction and/or polycystic ovarian morphology verified by ultrasound)

NIH – National Institutes of Health; ESHRE – European Society for Human Reproduction and Embriology;
ASRM – American Society for Reproductive Medicine; AES – Androgen Excess Society;
AES-PCOSS – Androgen Excess Society and PCOS Society

(Modified by reference Livadas S, Diamanti-Kanadarakis E, 2013)

Oxidative Stress and Inflammation



Prooxidants:

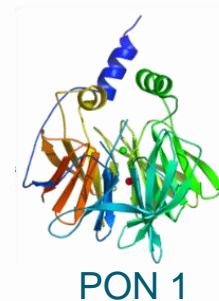
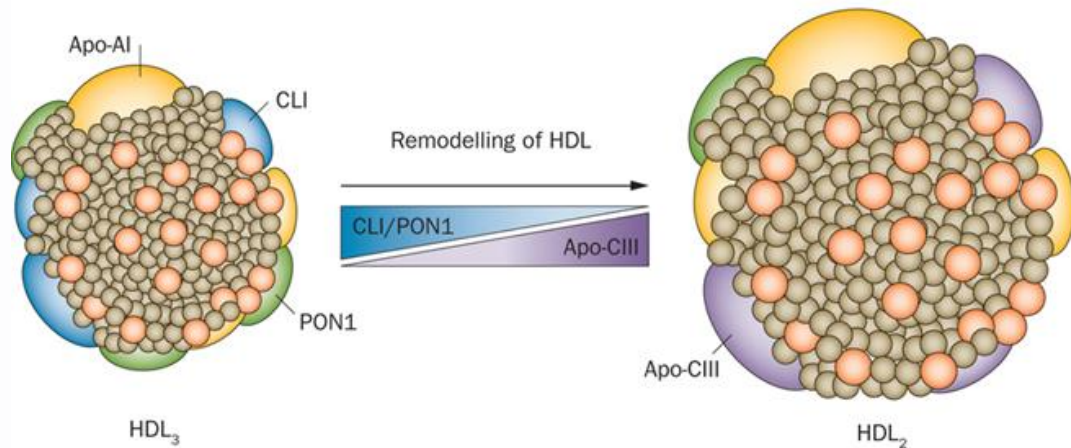
★ TOS, AOPP, PAB, MDA

Antioxidants:

★ TAS, SOD, PON 1, -SH groups

HDL Subclasses

Parameter	HDL 2b	HDL 2a	HDL 3a	HDL 3b	HDL 3c
Density (kg/L)	1.099	1.107	1.123	1.155	1.186
Size (nm)	10.4	10.3	9.9	8.0	7.3

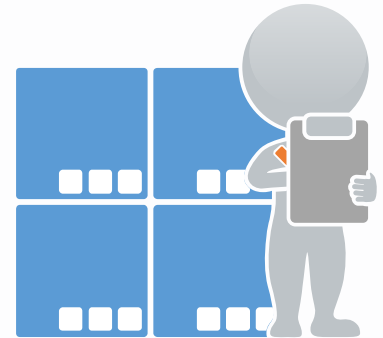




AIMS

Versus healthy women:

- ★ To determine the presence and type of dyslipidemia as well as the level of inflammation in PCOS patients
- ★ To determine if there are differences in oxidative stress and antioxidant protection parameters in PCOS patients
- ★ To determine PON 1 activity in PCOS patients
- ★ To determine the distribution of HDL lipoprotein subclasses in PCOS patients
- ★ To determine PON1 activity on HDL 2 and HDL 3 subclasses in PCOS patients





Materials and Methods

114 PCOS patients



18-39 years



50 healthy women

Diagnosis of PCOS Rotterdam (ESHRE/ASRM) /2003

In previous 6 months:

- ✓ Without oral contraceptives
- ✓ No antiandrogens
- ✓ No antihypertensive drugs
- ✓ No antidiabetic medication
- ✓ Without cigarettes and alcohol



- ✓ DBP \leq 90mm Hg and/or
- ✓ SBP \leq 140mm Hg
- ✓ Without oral contraceptives
- ✓ No antiandrogens
- ✓ No glucocorticoids
- ✓ No impaired glucose metabolism
- ✓ Without cigarettes and alcohol

Blood samples were collected in the morning after a 12h fasting period in the follicular phase of the cycle (on the 2nd or 3rd day) from both PCOS patients and the CG or randomly in the case of severe oligomenorrhea or amenorrhoea (in PCOS patients)

Oxidative stress parameters:

AOPP, TOS, PAB, MDA

Antioxidant protection parameters:

TAS, -SH groups, SOD

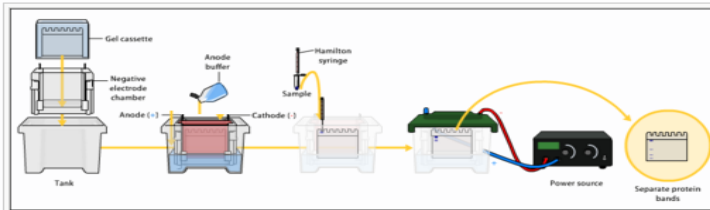
PON1 activity



Ilab 300+

Separation of HDL particles

PON 1 activity on HDL2 and HDL3 subclasses determination



Non-denaturing (3-31%) polyacrylamide gradient gel electrophoresis

Gradient gel electrophoresis



Trinder reaction



PON1 activity



Results

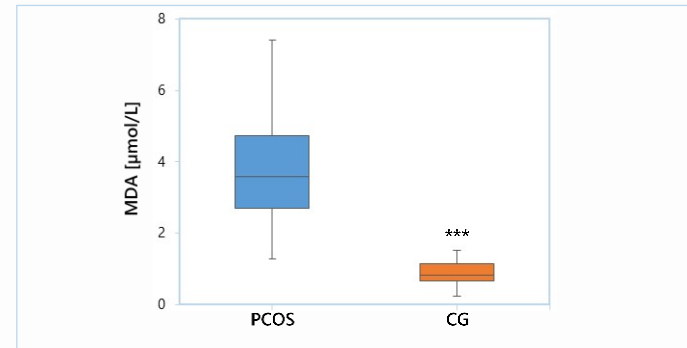
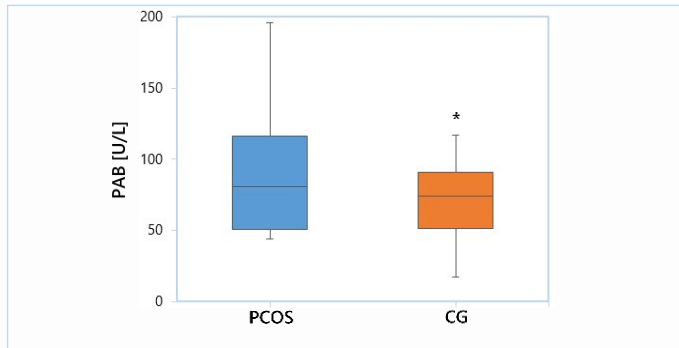
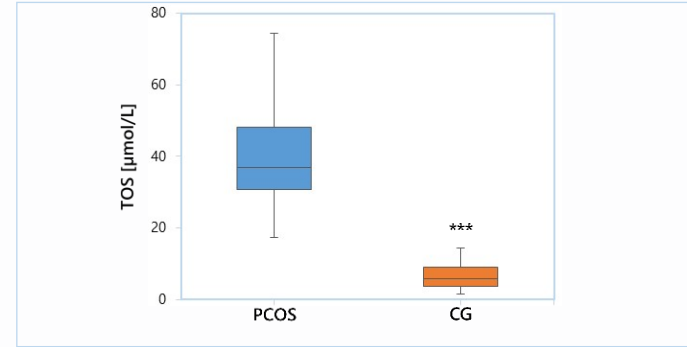
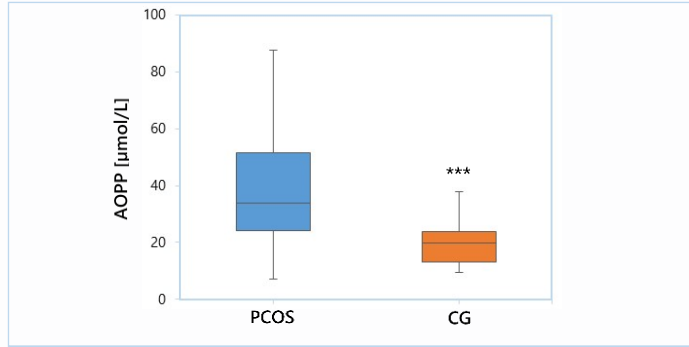


Demographic, Clinical and Biochemical Data in the Study Groups

Parameter	PCOS (n=114)	CG (n=50)	P
BMI, kg/m ²	24.2 (21.2–29.7)	22.1 (20.7–23.5)	<0.01
Age, years	25.5 (22.0–29.2)	28.0 (23.7–30.0)	0.177
SBP, mmHg	120 (110–125)	110 (103–120)	<0.01
DBP, mmHg	75.0 (70.0–80.0)	74.5 (65.7–80.0)	0.371
TC, mmol/L	4.76 (4.14–5.31)	4.98 (4.38–5.50)	0.232
LDL-C, mmol/L	2.84 (2.19–3.28)	2.81 (2.53–3.12)	0.789
HDL-C, mmol/L	1.40 (1.10–1.80)	1.60 (1.32–1.95)	<0.01
TG, mmol/L	0.840 (0.657–1.420)	0.930 (0.712–1.370)	0.745
Non-HDL-C, mmol/L	3.24 (2.57–3.93)	3.39 (2.87–3.76)	0.653
TG/HDL-C	0.644 (0.387–1.200)	0.503 (0.401–0.876)	0.173
CRP, mg/L	1.90 (0.70–3.70)	0.55 (0.40–1.00)	<0.001

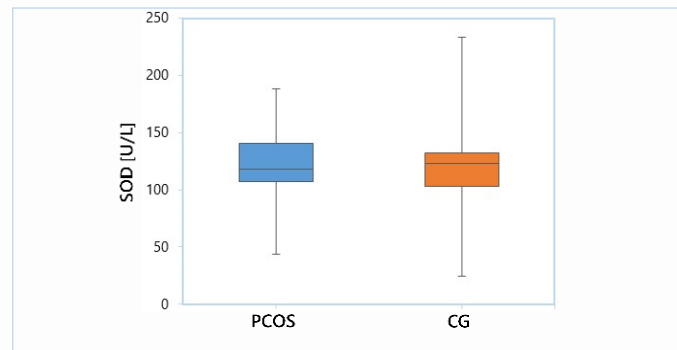
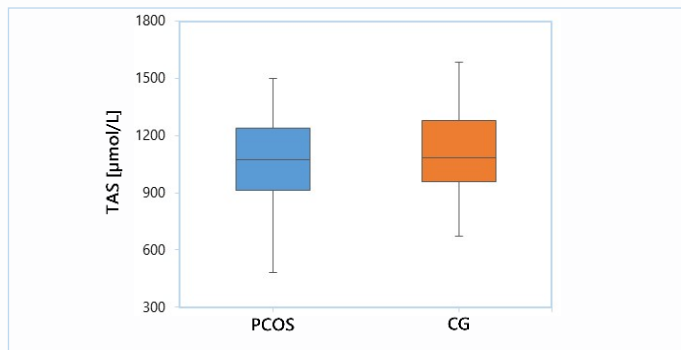
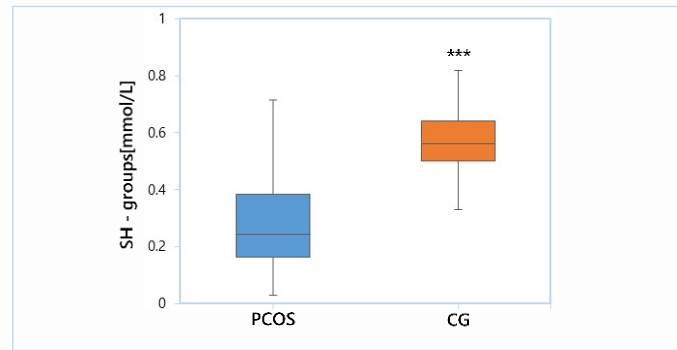
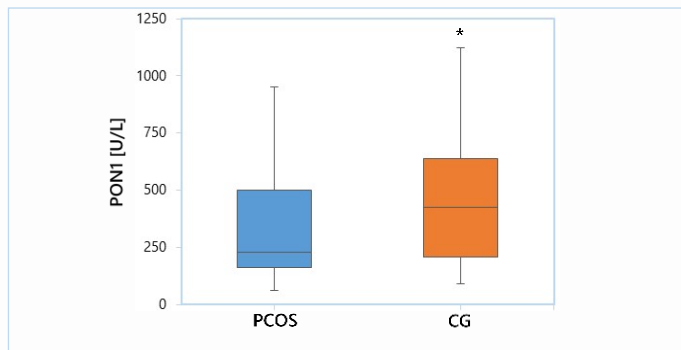


Redox Status - Prooxidants



*P<0.05, ***P<0.001 compared to PCOS patients, according to the Mann-Whitney U test

Redox Status - Antioxidants



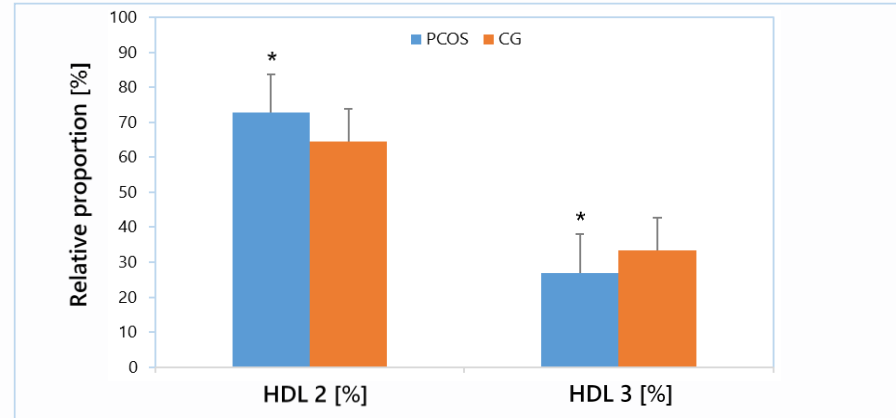
* $P < 0.05$, *** $P < 0.001$ compared to PCOS patients, according to the Mann-Whitney U test

HDL Subclasses Analysis

HDL subclasses particle size and distribution in the study groups

Parameter	PCOS (n=114)	CG (n=23)	P
HDL diameter, nm	10.44±0.87	10.24±0.99	0.381
HDL 2b, %	51.6±10.0	46.7±11.1	<0.05
HDL 2a, %	21.1±6.2	18.0±3.3	<0.01
HDL 3a, %	12.2±3.8	13.5±3.6	0.127
HDL 3b, %	6.4±3.2	9.1±3.6	<0.001
HDL 3c, %	8.5±4.1	11.0±4.4	<0.05

Relative proportions of HDL 2 and HDL 3 subclasses in the study groups



*P<0.05 compared to CG

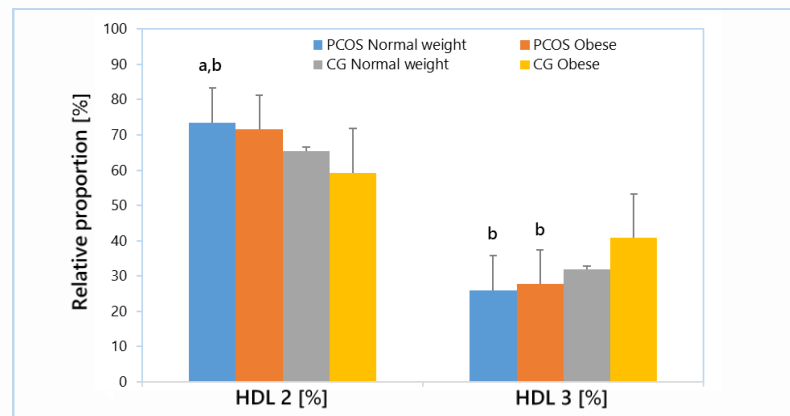


HDL Subclasses Analysis

HDL subclasses particle size and distribution in the study subgroups

Parameter	PCOS		CG		P
	Normal weight (BMI≤24.9 kg/m ²) (n=55)	Obese (BMI≥25.0 kg/m ²) (n=59)	Normal weight (BMI≤24.9 kg/m ²) (n=19)	Obese (BMI≥25.0 kg/m ²) (n=4)	
HDL-H, mmol/L	1.71±0.47 ^{bbb}	1.24±0.32	1.67±0.35 ^{bbb}	1.42±0.22	<0.001
HDL diameter, nm	10.62±0.79	10.24±0.91	10.03±0.90	11.37±0.61	<0.01
HDL 2b, %	52.8±10.6	50.3±9.3	47.7±10.8	41.7±13.2	0.066
HDL 2a, %	20.9±7.6	21.4±4.3	18.1±3.4	17.6±2.5	0.124
HDL 3a, %	11.5±3.3	13.1±4.2	13.3±3.2	14.6±5.8	0.058
HDL 3b, %	6.3±3.2 ^{aa}	6.5±3.2 ^{aa}	8.4±3.3	12.6±3.4	<0.001
HDL 3c, %	8.5±4.6	8.5±3.6	10.5±4.3	13.6±4.8	<0.05

Relative proportions of HDL 2 and HDL 3 subclasses in the study groups



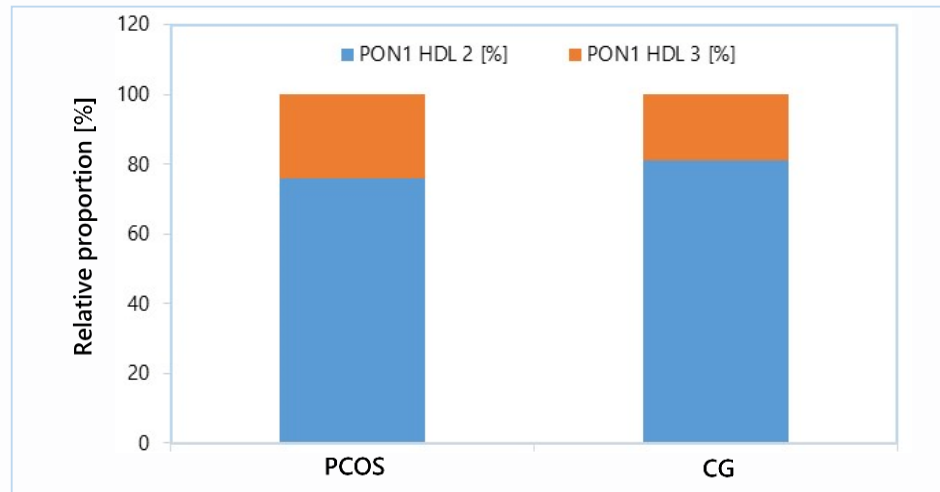
a – difference compared to normal weight CG; b – difference compared to obese CG.
P<0.05

PON 1 activity on HDL subclasses

PON 1 distribution on HDL subclasses
in the study groups

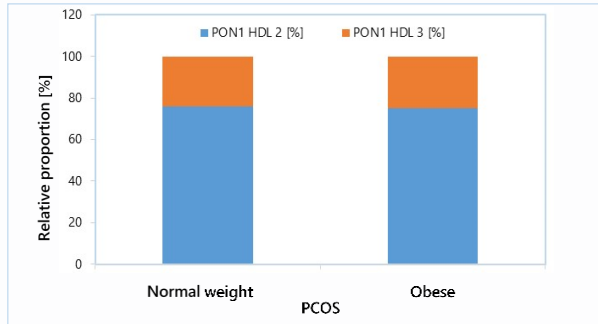
Parameter	PCOS	CG	P
PON1 HDL 2b, %	51.6±10.7	54.0±7.0	0.625
PON1 HDL 2a, %	24.1±5.7	26.6±2.8	0.348
PON1 HDL 3a, %	11.8±5.1	10.3±1.6	0.518
PON1 HDL 3b, %	6.4±3.2	4.9±1.7	0.324
PON1 HDL 3c, %	6.1±5.0	4.2±2.5	0.412

Relative proportions of PON 1 activity on HDL2 and HDL3 subclasses in the
study groups



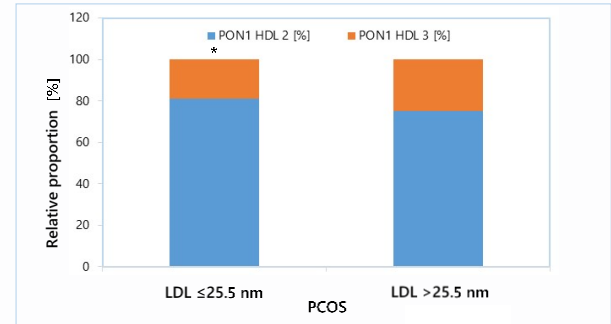
PON 1 activity distribution on HDL subclasses in PCOS subgroups

Parameter	PCOS		P
	Normal weight (BMI≤24,9 kg/m ²) (n=53)	Obese (BMI≥25,0 kg/m ²) (n=48)	
PON1, U/L	231 (145–527)	231 (176–472)	0.923
PON1 HDL 2b,%	51.8±11.4	51.4±10.0	0.837
PON1 HDL 2a,%	24.0±5.0	24.3±6.4	0.844
PON1 HDL 3a,%	11.8±5.8	11.8±4.3	0.949
PON1 HDL 3b,%	6.1±3.4	6.7±3.0	0.361
PON1 HDL 3c,%	6.2±4.9	5.9±5.1	0.765



PON 1 activity distribution on HDL subclasses in PCOS patients according to LDL particle size

Parameter	PCOS		P
	LDL diameter ≤25,5 nm (n=86)	LDL diameter >25,5 nm (n=15)	
PON1, U/L	313 (216–586)	233 (165–527)	0.279
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



*P<0.05



CONCLUSIONS

-
- ★ PCOS patients have increased oxidative stress and diminished antioxidative protection parameters.
 - ★ Chronic low-grade inflammation is been present in PCOS patients and indicates currently low cardiovascular risk.
 - ★ Normal weight PCOS patients have a significantly higher proportion of HDL2 subclasses compare to both control subgroups. Both PCOS subgroups have a lower proportion of HDL3 subclasses compare to the control group.



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- ★ Obesity has no influence on the distribution of PON 1 on HDL subclasses within the PCOS group, and there are no significant differences in the PON 1 distribution on HDL subclasses between PCOS patients and CG.
 - ★ Presence of sdLDL particles leads to the redistribution of HDL subclasses which is reflected through the reduced proportion of PON1 activity on HDL 3 subclasses.





Q&A
Thank you

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