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ASSESSMENT OF AORTIC WAVE REFLECTION IN LEAN AND OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Introduction. Conflicting findings have been published regarding the pressure wave reflection and the arterial stiffness in young women with polycystic ovary syndrome (PCOS) as opposed to the overt *ab initio* presence of endothelial dysfunction, which can be reversed six months after metformin administration.
The aim of this study was to investigate wave reflections in women with PCOS and to evaluate the effect of metformin treatment.

Résumé

L'évaluation de la réflexion des ondes aortiques chez des femmes maigres et obèses avec le syndrome des ovaires polykystiques

Introduction. Des résultats contradictoires ont été publiés concernant la réflexion des ondes de pression et la raideur artérielle chez les jeunes femmes atteintes du syndrome des ovaires polykystiques (SOPK), par opposition à la présence *ab initio* manifeste de la dysfonction

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Material and methods. Sixty-four young women, 35 with PCOS (P) (20 lean(L), P_L; 15 overweight/obese (OWB), P_{OWB}) and 29 controls (18 C_L;11 C_{OWB}) were studied. Wave reflection was assessed by the Augmentation Index (AIx) as central augmentation pressure-to-pulse height ratio corrected for heart rate (HR) 75 bpm (AIX@75) or without HR correction AIx and the central augmentation time index (Tr). The endothelial function was evaluated biochemically by plasma endothelin 1(ET-1) levels. The metabolic and hormonal profile and advanced glycated end-products (AGEs) levels were also assessed. Metformin (1700 mg/daily) was administered for six months in 20 (9 lean, 11 obese) women with PCOS and the measurements were repeated.

Results. All subgroups had comparable age. Wave reflection indices did not differ between PCOS and controls. AIx@75 significantly improved post-metformin treatment in P_{OWB} (p=0.046). AGE levels differed between PCOS women groups and controls ($p<0.001$), but their values became normal after metformin treatment. ET-1 levels did not differ between PCOS and controls, but they were significantly improved post-metformin treatment in both lean and obese PCOS groups (p=0.01, p=0.04, respectively).

Conclusions. Wave reflection markers seem to be a covert negative predictor in PCOS, which ameliorates after treatment with metformin, particularly in the overweight/obese subgroup of PCOS women.

Keywords: aortic wave reflection, insulin resistance, polycystic ovarian syndrome, metformin, endothelin-1.

List of abbreviations:

AIx: augmentation index (not corrected)
AIx@75: augmentation index corrected for heart rate 75 bpm
Tr: central augmentation time index
ET-1: endothelin 1
PWV: pulse wave velocity
FMD: flow-mediated dilatation
PCOS: polycystic ovary syndrome
OWB: overweight/obese women
L: lean women
P: polycystic ovary syndrome women
HR: heart rate
AGEs: advanced glycated end-products
BMI: body mass index
WHR: waist-to-hip ratio
IR: insulin resistance
OGTT: oral glucose tolerance test
SBP: systolic blood pressure
DBP: diastolic blood pressure
FAI: free androgen index
SHBG: sex hormone binding globulin
OCP: oral contraceptive pills

endothéliale, qui peut être inversée six mois après l'administration de la metformine.

L'objectif de cette étude était d'étudier les réflexions des ondes dans le SOPK et d'évaluer l'effet du traitement par la metformine.

Matériel et méthodes. Soixante-quatre jeunes femmes, 35 atteintes de SOPK (P) (20 maigres (L), PL; 15 en surpoids/ obèses (OWB), P_{OWB}) et 29 témoins (18 CL; 11 C_{OWB}) ont été étudiées. La réflexion des ondes a été évaluée par l'indice d'augmentation (AIx) en tant que rapport hauteur / pression d'augmentation centrale corrigé pour la fréquence cardiaque (HR) 75 bpm (AIX@75) ou sans correction HR AIx et l'index de temps d'augmentation centrale (Tr). La fonction endothéliale a été évaluée biochimiquement par les niveaux plasmatiques d'endothéline 1 (ET-1). Le profil métabolique et hormonal et les niveaux avancés de produits finaux glyqués (AGEs) ont également été évalués. La metformine (1700 mg/jour) a été administrée pendant six mois à 20 femmes (9 maigres, 11 obèses) atteintes de SOPK et les mesurages ont été répétés.

Résultats. Tous les sous-groupes avaient un âge comparable. Les indices de réflexion des ondes ne différaient pas entre le SOPK et les témoins. AIx@75 a significativement amélioré le traitement post-metformine dans le P_{OWB} (p=0.046). Les niveaux d'AGE différaient entre les groupes de femmes SOPK et les témoins ($p <0.001$) mais leurs valeurs ne se normalisaient pas après le traitement à la metformine. Les niveaux d' ET-1 ne différaient pas entre le SOPK et les témoins, mais le traitement post-metformine s'était significativement amélioré dans les groupes SOPK maigres et obèses (p=0.01, p=0.04, respectivement).

Conclusions. Les marqueurs de réflexion des ondes semblent être un prédicteur négatif caché dans le SOPK,ce qui améliore le traitement post-metformine en particulier dans le sous-groupe en surpoids / obèse des femmes SOPK.

Mots-clés: réflexion des ondes aortiques, résistance à l'insuline, syndrome des ovaires polykystiques, metformine, endothéline-1.

Introduction

Polycystic ovary syndrome (PCOS) is considered the most common endocrinopathy in women of reproductive age[1]. The fact that PCOS has been linked to metabolic disorders, such as overweight, insulin resistance, glucose intolerance, dyslipidemia, coagulopathy, as well as cardiovascular risk factors, such as chronic inflammation, or abnormal microvascular and macrovascular properties (assessed by haemodynamic or biochemical methods)[2-10], makes it a unique model for investigating the impact that these factors have on cardiovascular system.

Endothelial dysfunction is an early and well-studied cardiovascular risk factor in PCOS women, implying an overt *ab initio* endothelial dysfunction compared with normal women, which exhibit reversal after short-term metformin treatment[2,11]. Non-invasive techniques have been used to assess endothelial dysfunction, a marker of macrovascular function in conduit arteries, by using high-resolution ultrasonography on brachial artery, assessing endothelial-dependent, flow-mediated dilatation (FMD)[2,12], as opposed to microvascular function that has been assessed in resistance arteries, mostly by venous occlusion plethysmography[12]. The structural vascular properties, such as carotid arteries intima-media thickness, have been also studied with contradictory results[8,13]. On the other hand, another less studied cardiovascular risk factor, reflecting the functional vascular properties, is the arterial stiffness and pressure wave reflections, that are mostly assessed by the central pulse wave velocity (PWV) or by the assessment of augmentation index (AIx), with contradictory results in PCOS, up to date[14]. Original studies have shown that PCOS women exhibited stiffer arteries[15-17]. However, more recent studies demonstrate a similar arterial stiffness, AIx and PWV between controls and PCOS women[18,19]. Compliance and stiffness index were found impaired by ultrasonographic methods in 20 women with PCOS and 20 women with isolated polycystic ovarian morphology, compared to controls, but with different body mass index (BMI)[15]. In addition, impairment was also observed by recording PWV across the brachial artery in 19 women with PCOS versus 12 older control subjects with similar BMI and blood pressure[16]. The largest of these three studies demonstrated that 100 young, overweight/obese women with PCOS presented increased arterial stiffness assessed by increased central PWV compared to controls of similar age, BMI and waist-to-hip ratio (WHR), while stepwise regression in PCOS women showed that insulin resistance (IR) and lipids were independent predictors of PWV[17]. In addition, despite the conflicting data, advanced glycation end-products (AGEs) have been related to arterial stiffness[4] and increased AGE levels have been detected in young women with PCOS[12].

The aim of the study was to apply in our well-characterized PCOS population a simple and easily repetitive method to measure aortic wave reflection, namely the AIx, and investigate AGE levels after the subcategorization of our population in lean and obese women, in order to include obesity as a putative factor of the vascular abnormalities. Moreover, the effect of metformin in all these parameters was evaluated in a subset of the studied patients.

Material and methods

Subjects

The study consisted of 64 young women, 35 with PCOS (P) (20 lean (L), P_L; 15 overweight/obese (OWB), P_{OWB}), and 29 (18 C_L; 11 C_{OWB}) normal women recruited from previous studies[2,8,10]. The study protocol was approved by the local ethics board and informed consent was obtained from all participants[2,8,10].

The enrolled population was clinically healthy and did not suffer from any chronic or acute disease. Drugs that could interfere with the hormonal and metabolic studies (oral contraceptive pills (OCP), anti-inflammatory drugs) were discontinued for at least three months before enrollment in the study. Each patient with PCOS met the diagnostic criteria for PCOS, based on the Androgen Excess Society guidelines[21]. Secondary forms of PCOS[22], such as non-congenital adrenal hyperplasia, androgen secreting neoplasm, hyperprolactinemia, and thyroid disease were excluded before recruitment, by appropriate tests in the PCOS women[2,8,10]. In the control group, there was no hyperandrogenemia or evidence of clinical hyperandrogenism (hirsutism, acne or alopecia) on physical examination and no menstrual disturbances or subfertility problems reported[2,8,10].

Protocol

The metabolic profile and patients' follow-up were performed as previously described[10]. The evaluations were conducted within the follicular phase of menstrual cycle in control women and at any time in the PCOS women, who were chronically anovulatory. In the amenorrhoeic women, recent ovulation was excluded by progesterone measurement (<5 nmol/L) and chronic anovulation was assessed as oligomenorrhea, i.e. less than 8 cycles per year or menstrual cycle duration more than 35 days. Blood samples were collected on day 1, at 08:00 to 10:00 h after an overnight fast. After a 30-min resting period in the supine position, blood samples were collected

(time 0), followed by the oral glucose tolerance test (OGTT) post oral 75-g glucose load with blood samples obtained at 30-min intervals (30', 60', 90', 120'). Physical examination was performed in each person by two doctors[2,9,10]

The hemodynamic study was performed on day 2 in the Vascular laboratory. All measurements were performed in a quiet, temperature-controlled room, after an overnight 14-hour fast. All current smokers were requested to reduce the number of cigarettes for one-week before the hemodynamic study and to avoid smoking two days before the study[8].

Weight, height and WHR and BMI were measured[2]. Systolic (SBP) and diastolic (DBP) blood pressure were measured by a mercury sphygmomanometer, with the subject placed in a sitting position, after resting for at least 5 min. The average of three measurements was obtained. Lean women had a BMI ≤ 25 kg/m^2 and overweight/obese women had a BMI > 25 kg/m^2.

Metformin protocol

After baseline measurements, metformin was administered in women with PCOS for six months[2]. Twenty women with PCOS received a daily dose of 1700 mg for 6 months (Lipha Sante, Aron Medicia Division, Lyon, France). No severe side effects were reported during the study. However, two women reported flatulence and they were recommended to reduce the dose of metformin by 850/2 mg for a week, afterwards they maintained the full dose until the end of the study. Upon completion of 6 months treatment, the hemodynamic, hormonal and metabolic measurements were repeated. A close follow-up of PCOS women was performed for the entire study period.

Hemodynamic studies

Aortic hemodynamics were assessed non-invasively, using the technique of radial artery tonometry and pulse wave analysis (Sphygmocor System-Atcor Medical, Sydney, Australia). Peripheral pressure waveforms were recorded at the radial artery using a hand-held high-fidelity tonometer (Millar, Instruments, Houston, TX, USA). The recorded pulse waves were calibrated using the SBP and DBP values measured at the brachial artery. Aortic pressure waveforms were then mathematically derived by applying generalized transfer functions[24]. Pulse wave analysis of the aortic waveform was used to calculate indices that correspond mainly to measures of arterial and aortic stiffness especially and to the intensity of reflected waves. The AIx(%) was calculated as the ratio of augmented pressure (pressure at the second inflection point minus pressure at the first inflection point of the systolic part of pressure waveform) to pulse pressure. In addition, timing of the reflected wave (Tr), which is a surrogate of pulse wave velocity, was determined as the time between the first foot of the pressure wave and the inflection point, indicating the arrival of the reflected wave at central aorta[25]. The Aix, which is influenced by heart rate[26,27], was also normalized for heart rate at 75 bpm (AI@75).

Blood assays

Blood samples were centrifuged immediately, and serum was stored at –20°C until assayed[2,8].

Serum endothelin-1 levels (ET-1, fmol/mL) were assessed[2]. AGE levels (U/mL) were estimated by competitive AGE-ELISA procedure[27].

Insulin resistance estimation

Insulin resistance was estimated by the Quantitative Insulin sensitivity Check Index (QUICKI) and Matsuda Index.

QUICKI is defined as:

$$QUICKI = 1/ [\log (Fasting\ Insulin) + \log (Fasting\ Glucose)]$$[28].

MATSUDA INDEX is obtained using the following formula:

$$MATSUDA\ (M) = 10.000 / \text{square root of } [(fasting\ glucose \times fasting\ insulin) \times (mean\ glucose \times mean\ insulin\ during\ OGTT)]$$[21].

Hyperandrogenemia estimation

The hyperandrogenemic index (free androgen index, FAI, %) was estimated by the formula:

FAI= [TT (nmol/L)/SHBG (nmol/L)] x 100 (%)[22]

Statistical analysis

Results are reported as median value with interquartile range. Statistical significance was accepted at a p value < 0.05. Mann-Whitney U was used for comparisons between PCOS women and the control group and Wilcoxon t-test were applied to evaluate changes between measurements at baseline and after the six months treatment period. Categorical variables were assessed by chi-square test corrected by Fisher's exact test when appropriate and exact test correction as appropriate. The analysis was performed using SPSS (version 22.0; PASW SPSS, Inc., Chicago, IL, USA).

Results

Demographic profile

The age did not differ between the study groups. BMI differed in lean PCOS versus lean control women, but did not differ between obese women. BMI and WHR remained unchanged after metformin therapy (Table 1) and no adjustment for BMI was

Table 1. The parameters studied in obese PCOS (P_{OB}) and lean PCOS (P_L) patients pre-metformin (pre-M) normal obese (C_{OB}) and lean (C_L) control women and their comparisons and the comparison between the overweight/obese and the lean groups, respectively, was performed by Mann Whitney U test. The pre-metformin and post-metformin (post-M) PCOS were compared by Pair Wilcoxon test and the comparison was made between 11 and 9 paired of overweight/obese and lean PCOS women, respectively.

Variables Studied Conventional units [SI units]	*Studied Groups*			*Studied Groups*		
	P_{OB}*pre-M (n=20) 0 20)*	P_{OB}*post-M (n=11)*	C_{OB} *(n=11)*	P_L*pre-M (n=15) 0 20)*	P_L*post-M (n=9)*	C_L *(n=18)*
Age (years)	27 (10,18-36)	25 (7, 21-32)	25 (10, 20-40)	25 (6, 17-35)	25 (7, 21-32)	28.5 (4,20-34)
BMI (kg/m^2)	33.5 (8.8,26.9-46.1)	34.8(7.7,28.3-37.3)	29.9 (8.9,27.1-40.2)	22.6 (4.9,16.5-25.9)[b]	22 (6.2,16.8-27.4)	20.5(3.1,16.3-25.2)
WHR (cm)	0.82 (0.07, 1.25-0.88)	0.82 (0.13, 0.74-0.9)	0.77 (0.13, 0.71-0.91)	0.74 (0.13, 0.66-0.90)	0.75 (0.14, 0.66-0.87)	0.72 (0.06, 0.63-0.83)
TT (ng/dL, nmol/L)	88.5 (29, 36-154)[a] [3.1 (1, 1.25-5.34)]	54 (34.8, 23.9-100)[a,c] [1.87, (1.21, 0.83-3.47)]	34.2 (19, 22-60) [1.19 (0.66, 0.76-2.1)]	71 (74, 33-148)[b] [2.46 (2.57, 1.14-5.13)]	54 (36.5, 36-170)[b] [1.87 (1.27, 1.25-5.89)]	34.3 (24, 12-72) [1.19 (0.83, 0.42-2.5)]
SHBG (nmol/L)	29.2 (24, 15-92)	25 (13, 18-37)	31 (15, 21-60)	35 (26, 13-60)	46 (40, 14-70)	41.5 (31, 29-90)
FAI index (%)	305.3 (299.2, 91.5-812.5)[a]	226.1 (90, 89-270)[a]	112.4 (48.6, 37.3-285.7)	216.9 (516,94.3-984.6)[b]	144 (212, 57-1206)[b]	82.6 (61.1, 28.5-217.2)
Glucose (mg/ dL, mmol/L)	80 (23, 70-121) [4.44 (1.28, 3.89-6.72)]	83.5 (25, 76-106) [4.63 (1.39, 4.22-5.88)]	83 (18, 69-108) [4.61 (1, 3.83-5.99)]	83 (20, 65-104) [4.61, (1,11 (3.61-5.77)]	81 (15, 70-94) [4.4 (0.83, 3.89-5.22)]	80 (12, 70-103) [4.44 (0.67, 3.89-5.72)]
INS (μU/mL, pmol/L)	11.7 (27, 6-88) [81.25 (187.5, 41.67-611.11)]	11 (14.1, 6-30.8) [76.39 (97.92, 41.67-213.89)]	10 (6, 6-26) [69.44 (41.67, 41.67-180.56)]	7.6 (4, 4-14) [52.78 (27.78, 27.78-97.22)]	9.7 (13.9, 4.5-22.1) [67.36 (96.53, 31.25-153.47)]	6 (2, 4-10) [41.67 (13.89-69.44)]
GIR	6.7 (7, 1-12)	8.8 (8.3, 3.3-13.1)[a]	8.3 (5, 3-14)	10.7 (7, 5-26)	8.1 (10, 3.3-18)	13.2 (6, 8-22)
HOMA	2.5 (4.4, 1.1 – 19.4)	2.4 (3.5, 1.2 – 7.8)	2.1 (1.8, 1.2-5.5)	1.4 (1.2, 1 – 2.6)	2.1 (2,8, 0.9– 4.9)	1.2 (0.7, 0.7-2)
QUICKI	0.33 (0.05, 0.26 – 0.38)	0.33 (0.07, 0.29-0.37)	0.34 (0.04, 0.30-0.37)	0.36 (0.04, 0.33-0.38)	0.34 (0.07, 0.30-0.39)[b]	0.37 (0.03, 0.34-0.4)
MATSUDA	2.6 (2.3, 0.5-5.9)[a]	2.9 (1.6, 1.5-4.8)[a]	4.6 (3.9, 2.5-8.3)	3.8 (2.9, 1.7-8.7)[b]	2.6 (2.1, 1.6-6.9)[b]	8.4 (4.8, 3.1-18.5)
SBP (mmHg)	117.5 (21, 85-140)	127.5 (36, 100-140)	110 (10, 90-125)	110 (15, 95-135)	100 (20, 80-125)	110 (23, 90-130)
DBP (mmHg)	80 (25, 50-95)	80 (15, 60-90)	80 (15, 50-85)	70 (10, 60-90)	65 (23, 60-90)	65 (16, 55-80)
ET-1 (fmol/mL)	7.4 (4.4, 1.2-10.8)	4.9 (5.1, 1.1-6.8)[c]	6.1 (5.5, 1.2-9.1)	7.3 (3.9, 3.3-11.2)	1.4 (3, 1.1-6.9)[d]	6.1 (5.8, 1.2-9.7)
AGES (U/mL)	9.9 (0.9, 5.8-11)[a]	10.2 (1.48.9-10.8)[a]	5 (0.8, 4.6-6)	9.9 (1.3, 9-11.2)[b]	9.9 (0.8, 9.1-11)[b]	5 (0.8, 4.3-9)
AIx	15 (17, –16-40)	10.5 (18, 1-42)	13 (6, –7-31)	10.5 (15, –12-26)	3 (18, –2-22)	14 (11, –5-38)
AIX@75	16 (17,-11- 39)	13 (16, 5-34)[c]	15(10,-3- 29)	12.5 (16, –9-22)	15 (14, 0-27)	11.5 (11,-1- 35)
Tr	117.5 (22,84-166)	112 (28, 101-172)	116(8, 93-144)	112 (19, 88-136)	103 (22, 98-128)	116.5 (17, 95-162)

Data as median (IQR, range); P<0.05 as statistically significant; [a]vs C_{ob}; [b]vs C_L;[c]vs P_{OB}preM; [d]vs $P_L$preM

performed. The subgroups studied did not differ in age, smoking habits or positive family history for type 2 diabetes mellitus.

Wave reflection estimation

AIx@75 and Tr did not differ between PCOS and controls; AIx@75 was significantly improved after metformin treatment in P_{OWB} (p=0.046), but not in P_L (p=0.89).

ET-1 levels

ET-1 levels did not differ between obese or lean PCOS and controls (p=0.29 and p=0.28, respectively), but they were significantly improved post-metformin treatment in both lean and obese PCOS groups (p=0.01 and p=0.04, respectively). ET-1 levels dropped significantly post-treatment in both P_{OWB} (p=0.04) and P_L (p=0.01).

Advanced glycated end-products

AGEs differed between PCOS and controls in both lean or obese (p<0.001). However, no difference was observed post-treatment in both P_{OWB} (p=0.10) and P_L (p=0.52).

Hormonal and metabolic parameters estimation

Testosterone levels were higher in PCOS compared to control women (p=0.003 in P_L vs. C_L and p<0.001 P_{OWB} vs. C_{OWB}) and decreased post-metformin (p=0.003 in P and p=0.008 in C), but without reaching control levels (p=0.001 P_L post-M vs. C_L and p<0.001 P_{OWB} post-M vs. C_{OWB}). Similarly, FAI had higher levels in PCOS compared to control women (p<0.001 in P_L vs. C_L and p=0.001 P_{OWB} vs. C_{OWB}), but SHBG levels did not differ between groups.

Fasting insulin and glucose levels, as well as systolic and diastolic pressure, did not differ between groups and did not change post-treatment.

Only MATSUDA index values were lower in PCOS compared to controls (P_L vs. C_L, p=0.004, P_{OWB} vs. $C_{OWB,}$ =0.02, respectively), but they did not normalize post-treatment.

Correlations

In all subgroups studied, AIx, AIx@75 and Tr correlated only with age (r=0.43, p=0.001, r=0.473, p<0.001, r=0.43, p=0.001, respectively). AGE levels did not correlate with aortic hemodynamic indices but with testosterone levels (r=0.78, p<0.001), FAI (r=0.70, p<0.001), SHBG (r=-0.38, p=0.009), DBP (r=0.31, P=0.04), MATSUDA index (r=-0.38, p=0.014). In the total PCOS population, AIx, AIx@75 and Tr, all three correlated only with age (r=0.45, p=0.01, r=0.60, p<0.001, r=0.44, p=0.01, respectively). AGE levels did not correlate with arterial stiffness hemodynamic indices but only with testosterone levels (r=0.45, p=0.02). In lean PCOS population, AIx@75 correlated only with age (r=0.89, p<0.001). In obese PCOS population, AGE levels correlated only with testosterone levels (r=0.62, p=0.008).

Discussion

In the present study, we have demonstrated that only a covert impairment of aortic wave reflection is present in a small group of overweight/obese women with PCOS, which can be documented by wave reflection improvement post-metformin treatment. This finding may explain the fact that there are contradictory results in the literature, as opposed to endothelial dysfunction that is an overt and early cardiovascular risk factor present in almost all the published studies. Arterial stiffness was also evidenced by increased AGE levels in PCOS women compared to controls, but this impairment did not apparently normalize post-metformin treatment.

Altered arterial stiffness and wave reflection have been previously investigated either versus normal women or versus PCOS[15-17,31-35]. In a large population of 84 women with PCOS and 95 healthy volunteers, central arterial stiffness and diastolic dysfunction were not increased in young women with PCOS, after adjustment for age and BMI, whereas it was associated with insulin resistance and abdominal obesity[36]. In parallel with this latter study, other researchers have not detected any difference in the afore mentioned indices between PCOS and control women[14,37-41]. These differences may be attributed to the small number of participants, the failure to correct for obesity, age, arterial pressure, cardiac rhythm, and particularly to the different methods used to estimate arterial stiffness or wave reflection and compliance along with the different arterial studies (either peripheral or central).

On the other hand, the improvement in wave reflection post-metformin treatment seen in the present study has been observed in most of the published studies, either in the case of single metformin administration or in combination with OCP[42-44]. The improvement in arterial stiffness has been attributed to the deterioration of insulin resistance of the group that did not receive metformin, following OCP treatment[42]. However, metformin did not improve AGE levels in PCOS women, as we have previously shown in a larger sample study with both lean and overweight/obese women[10]. Hence, only overt and early cardiovascular factors can always be seen in a young population like the pre-menopausal women with PCOS, but we have always to consider the presence of other covert or subclinical cardiovascular risk factors.

In parallel with wave reflection findings, ET-1 plasma levels did not show any differences between PCOS and control women, in either lean or overweight/obese groups, but metformin treatment significantly reduced ET-1 expression to lower levels than those of the respective control groups. The improvement of both endothelial dysfunction and wave reflection post-metformin administration was not followed by a parallel improvement in insulin resistance indices. Only in the obese subgroup, metformin improved the hyperandrogenic profile, as it has been shown in several previous studies[45].

The novelty of the present work relies on the fact that wave reflection was assessed in a well-studied population. Nonetheless, the sub-categorization based on the weight of PCOS and control women, unveiled subtle differences that are present before and after treatment, that may not be clear when lean and obese women are combined in a single group. It is important to identify the early markers of cardiovascular risks, since the impairment involves not only the young patients studied, but their siblings as well[46,47]. The emerged limitation relies to the small size of the studied population, that was unable to reveal differences in parameters that were altered in previous studies.

Conclusion

In conclusion, our study demonstrates that PCOS women display some overt and some covert cardiovascular risk factors. In a routine clinical practice, the usage of parameters that are non-invasive and easily reproducible may guide clinicians to an early intervention on these cardiovascular risk factors, by either life-style changes or pharmaceutical agents' administration.

Author Contributions:

Conceptualization, K.I.A, E.K., T.G.P; methodology, K.I.A, E.K., T.G.P, C.P.; software, T.G.P; validation, A.A.A., K.S., A.P., T.K.; formal analysis, K.I.A, E.K., T.G.P; investigation, K.I.A, E.K., T.G.P, A.P. ; resources, K.I.A.; data curation, K.I.A, E.K., T.G.P.; writing–original draft preparation, K.I.A, T.G.P.; writing–review and editing, C.P., K.S., A.P., J.L.; visualization, T.G.P.; supervision, A.P., J.L.; project administration, J.L. K.I.A., E.A.K., and T.G.P. contributed equally to this work. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. The informed consent has been obtained from all the patients included in the study."

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References

1. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *The Journal of Clinical Endocrinology and Metabolism.* 1999;84:4006-4011.
2. Diamanti-Kandarakis E, Alexandraki K, Protogerou A, et al. Metformin administration improves endothelial function in women with polycystic ovary syndrome. *European Journal of Endocrinology.* 2005;152:749-756.
3. Bernasconi D, Del Monte P, Meozzi M, et al. The impact of obesity on hormonal parameters in hirsute and non-hirsute women. *Metabolism.* 1996;45:72-75.
4. Rosenbaum D, Haber RS, Dunaif A. Insulin resistance in polycystic ovary syndrome: decreased expression of GLUT-4 glucose transporters in adipocytes. *American Journal of Physiology.* 1993;264:E197-E202.
5. Diamanti-Kandarakis E, Mitrakou A, Raptis S, Tolis G, Duleba AJ. The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism.* 1998;83:2699-2705.
6. Mannerås-Holm L, Baghaei F, Holm G, et al. Coagulation and fibrinolytic disturbances in women with polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism.* 2011;96:1068-1076.
7. Diamanti-Kandarakis E, Paterakis T, Alexandraki K, et al. Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. *Human Reproduction.* 2006;21:1426-1431.
8. Alexandraki K, Protogerou AD, Papaioannou TG, et al. Early microvascular and macrovascular dysfunction is not accompanied by structural arterial injury in polycystic ovary syndrome. *Hormones (Athens).* 2006;5:126-136.
9. Diamanti-Kandarakis E, Alexandraki K, Piperi C, et al. Inflammatory and endothelial markers in women with polycystic ovary syndrome. *Eur Journal of Clinical Investigation.* 2006;36:691-697.
10. Diamanti-Kandarakis E, Alexandraki K, Piperi C, et al. Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome. *Metabolism.* 2007;56:129-134.
11. Diamanti-Kandarakis E, Spina G, Kouli C, Migdalis I. Increased endothelin-1 levels in women with polycystic ovary syndrome and the beneficial effect of metformin therapy. *The Journal of Clinical Endocrinology and Metabolism.* 2001;86:4666-4673.
12. Corretti MC, Anderson TJ, Benjamin EJ; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the

International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology.* 2002;39:257-265.
13. Carmina E, Orio F, Palomba S, et al. Endothelial dysfunction in PCOS: role of obesity and adipose hormones. *American Journal of Medicine.* 2006;119:356.e1-6.
14. Cussons AJ, Watts GF, Stuckey BG. Dissociation of endothelial function and arterial stiffness in nonobese women with polycystic ovary syndrome (PCOS). *Clinical Endocrinology (Oxford).* 2009;71:808-814.
15. Lakhani K, Seifalian AM, Hardiman P. Impaired carotid viscoelastic properties in women with polycystic ovaries. *Circulation.* 2002;106:81-85.
16. Kelly CJ, Speirs A, Gould GW, Petrie JR, Lyall H, Connell JM. Altered vascular function in young women with polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism.* 2002;87:742–746.
17. Meyer C, McGrath BP, Teede HJ. Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. *The Journal of Clinical Endocrinology and Metabolism.* 2005;90:5711–5716.
18. Kim J, Choi SY, Park B, et al. Arterial stiffness measured by cardio-ankle vascular index in Korean women with polycystic ovary syndrome. *Journal of Obstetrics and Gynecology.* 2019;39:681-686.
19. Burlá M, Cunha AR, Gismondi R, Oigman W, Fritsch NM, Medeiros F. Endothelial dysfunction and pulse wave reflection in women with polycystic ovarian syndrome. *International Journal of Cardiovascular Sciences.* 2019;32:3-9.
20. Papaioannou TG, Alexandraki KI, Karamanou M, Piperi C, Tousoulis D. Association of skin autofluorescence with arterial properties: A closer look at AGE Reader and EndoPAT 2000 commercial devices. *Experimental Gerontology.* 2017;98:207-208
21. Azziz R, Carmina E, Dewailly D, et al; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The androgen excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertility and Sterility.* 2009;91:456-488.
22. Alexandraki KI, Kaltsas GA. Endocrinopathies and other disorders inducing a polycystic ovary syndrome phenotype. *Frontiers of Hormone Research.* 2013;40:142–157.
23. Papaioannou TG, Protogerou AD, Stamatelopoulos KS, Vavuranakis M, Stefanadis C. Non-invasive methods and techniques for central blood pressure estimation: procedures, validation, reproducibility and limitations. *Current Pharmaceutical Design.* 2009;15:245-253.
24. Papaioannou TG, Karatzis EN, Papamichael CM, et al. Circadian variation of arterial pressure wave reflections. *American Journal of Hypertension.* 2006;19:259-263.
25. Kyrlagkitsis S, Papaioannou TG, Gialafos E, et al. Relationships between heart rate variability and aortic hemodynamic variables in healthy subjects. *Hellenic Journal of Cardiology.* 2016;57:359-362.
26. Papaioannou TG, Vlachopoulos CV, Alexopoulos NA, et al. The effect of heart rate on wave reflections may be determined by the level of aortic stiffness: clinical and technical implications. *American Journal of Hypertension.* 2008;21:334-340.
27. Diamanti-Kandarakis E, Piperi C, Kalofoutis A, Creatsas G. Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. *Clinical Endocrinology (Oxford).* 2005;62:37–43.
28. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *The Journal of Clinical Endocrinology and Metabolism.* 2000;85:2402-2410.
29. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* 1999;22:1462–1470.
30. Clark AF, Marcellus S, deLory B, Bird CE. Plasma testosterone free index: a better indicator of plasma androgen activity? *Fertility and Sterility.* 1975;26:1001-1005.
31. Soares GM, Vieira CS, Martins WP, et al. Increased arterial stiffness in nonobese women with polycystic ovary syndrome (PCOS) without comorbidities: one more characteristic inherent to the syndrome? *Clinical Endocrinology (Oxford).* 2009;71:406-411.
32. Kaya MG, Gunebakmaz O, Zencir C, et al. An assessment of the elastic properties of the aorta in nonobese women with polycystic ovary syndrome. *Fertility and Sterility.* 2010;94:2402–2405.
33. Dessapt-Baradez C, Reza M, Sivakumar G, et al. Circulating vascular progenitor cells and central arterial stiffness in polycystic ovary syndrome. *PLoS One.* 2011;6:e20317.
34. Gencer M, Gazi E, Hacıvelioğlu S, et al. The relationship between subclinical cardiovascular disease and lipocalin-2 levels in women with PCOS. *European Journal of Obstetrics & Gynecology and Reproductive.* 2014;181:99–103.
35. Dargham SR, Ahmed L, Kilpatrick ES, Atkin SL. The prevalence and metabolic characteristics of polycystic ovary syndrome in the Qatari population. *PLoS One.* 2017;12:e0181467.
36. Rees E, Coulson R, Dunstan F, et al. Central arterial stiffness and diastolic dysfunction are associated with insulin resistance and abdominal obesity in young women but polycystic ovary syndrome does not confer additional risk. *Human Reproduction.* 2014;29:2041-2049.
37. Zueff LF, Martins WP, Vieira CS, Ferriani RA. Ultrasonographic and laboratory markers of metabolic and cardiovascular disease risk in obese women with polycystic ovary syndrome. *Ultrasound in Obstetrics and Gynecology.* 2012;39:341-347.
38. Moran LJ, Cameron JD, Strauss BJ, Teede HJ. Vascular function in the diagnostic categories of polycystic ovary syndrome. *Human Reproduction.* 2011;26:2192–2199.
39. Ketel IJ, Stehouwer CD, Henry RM, et al. Greater arterial stiffness in polycystic ovary syndrome (PCOS) is an obesity – but not a PCOS-associated phenomenon. *The Journal of Clinical Endocrinology and Metabolism.* 2010;95:4566-4575.
40. Muneyyirci-Delale O, Winer N, Oklander V, et al. Vascular compliance in women with polycystic ovary syndrome and healthy women. *Journal of the Cardiometabolic Syndrome.* 2007;2:40-44.
41. Trakakis E, Balanika A, Baltas C, et al. Hemodynamic alterations and wall properties in large arteries of young, normotensive, and non-obese women with polycystic ovary syndrome. *Journal Endocrinological Investigation.* 2008;31:1001–1007.
42. Meyer C, McGrath BP, Teede HJ. Effects of medical therapy on insulin resistance and the cardiovascular system in polycystic ovary syndrome. *Diabetes Care.* 2007;30:471-478.
43. Agarwal N, Rice SP, Bolusani H, et al. Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: a randomized, placebo-controlled, crossover trial. *The Journal of Clinical Endocrinology and Metabolism.* 2010;95:722-730.
44. Kaya MG, Calapkorur B, Karaca Z, et al. The effects of treatment with drospirenone/ethinyl oestradiol alone or

in combination with metformin on elastic properties of aorta in women with polycystic ovary syndrome. *Clinical Endocrinology. (Oxford)* 2012;77:885-892.
45. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. *European Journal of Endocrinology.* 1998;138:269-274.
46. Diamanti-Kandarakis E, Alexandraki K, Bergiele A, Kandarakis H, Mastorakos G, Aessopos A. Presence of metabolic risk factors in non-obese PCOS sisters: evidence of heritability of insulin resistance. *Journal Endocrinological Investigation.* 2004;27:931–936.
47. Sam S, Coviello AD, Sung YA, Legro RS, Dunaif A. Metabolic phenotype in the brothers of women with polycystic ovary syndrome. *Diabetes Care.* 2008;31:1237–1241.