

Research paper

Early microvascular and macrovascular dysfunction is not accompanied by structural arterial injury in polycystic ovary syndrome

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ABSTRACT

OBJECTIVE: During the last decade cardiovascular risk factors and endothelial dysfunction have been shown to be present early in life in women with Polycystic Ovary Syndrome (PCOS). The aim of the present study was a global assessment of abnormalities in the arterial bed of young women with PCOS by non-invasive, reproducible methods. **DESIGN:** 27 women with PCOS and 27 control women of comparable age, body mass index and waist-to-hip ratio were studied. Macrovascular function was assessed by flow-mediated dilatation (FMD) on the brachial artery. Nitrate-induced dilatation (NID) was performed to exclude a vascular smooth muscle cells injury. Microvascular function was assessed by venous occlusion plethysmography studying forearm blood flow. Arterial structure was evaluated by ultrasonographic assessment of intima-media thickness (IMT) of the carotid artery. **RESULTS:** FMD values were lower in women with PCOS compared to controls (PCOS: $3.84 \pm 0.74\%$ vs. controls: $9.83 \pm 0.97\%$, $P < 0.001$), but no difference was observed in NID (PCOS: $16.59 \pm 1.84\%$ vs. controls: $16.64 \pm 2.05\%$, $P = 0.98$) values. The time required for reactive hyperemia to reach peak value, a plethysmography parameter, was longer in PCOS women (PCOS: 20.63 ± 4.67 sec vs. controls: 10.38 ± 5.11 sec, $P = 0.02$). No difference was observed in the combined IMT among the studied groups (PCOS: 0.49 ± 0.01 mm v.s. controls: 0.51 ± 0.02 mm, $P = 0.19$). **CONCLUSIONS:** Using non invasive methodologies endothelial dysfunction in the macrocirculation and early impairment in the microcirculation were demonstrated in young women with PCOS who had normal profile of glycemia, lipidemia and blood pressure, and no evidence of structural arterial impairment.

Key words: Endothelial dysfunction, FMD, IMT, Insulin resistance, NID, PCOS, Plethysmography, Polycystic ovary syndrome

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy of women of reproductive age,^{1,2} and is characterized by hyperandrogenemia and chronic anovulation. Furthermore, PCOS is considered a potential risk factor for diabetes^{3,4} and cardiovascular disease.⁵⁻⁷ During the last decade, the impact of PCOS on the cardiovascular system has been extensively evaluated, focusing on endothelial disorders and structural changes in young women with this syndrome.

There are, however, conflicting data about endothelial function and arterial structure in young women with PCOS.⁸⁻¹⁷ The available data are difficult to compare because the populations studied are heterogeneous, carrying different cardiovascular risk factors, and not always matched with appropriate control groups.

Another important issue concerns the methods used to evaluate arterial characteristics. Laborious and invasive methods have been used to estimate vascular function,^{9,11,15,18} which bear not only an economic but also a psychological cost, particularly in populations of young women afflicted with PCOS.

It is to be stressed that micro- and macrocirculation may be differently affected by cardiovascular risk factors¹⁹ and may have complementary roles with regard to the nosology of the cardiovascular system.¹⁹ In the present study, a global assessment of the arterial bed, involving both the macro- and microcirculation, was undertaken in young women with PCOS, who had a normal profile of glycemia, lipidemia and blood pressure and comparable to the control group.

METHODS

Subjects

The study involved 54 women. Twenty-seven women with PCOS [Mean value \pm Standard Error (\pm SE)] [age: 25.41 ± 0.80 years; body mass index (BMI): 27.42 ± 1.12 kg/m²] were recruited from the Outpatient Section of Endocrinology of the 1st Department of Internal Medicine of the Laiko University Hospital in Athens. The diagnosis of PCOS was based on the presence of irregular menstrual

cycles (8 or fewer menses per year), elevated plasma levels of total testosterone and clinical signs of hyperandrogenemia, as suggested by the criteria of the National Institute of Child Health and Human Development conference (1990). Non-classical congenital adrenal hyperplasia, androgen-secreting neoplasm, hyperprolactinemia and thyroid disease were excluded by appropriate tests. Twenty-seven healthy women (doctors and medical students) [Mean value \pm Standard Error (\pm SE)] (age: 27.33 ± 0.83 years; BMI: 25.05 ± 1.19 kg/m²), matched for age, body mass index (BMI) and waist-to-hip ratio (WHR), volunteered to participate in the study and served as the control group. In the control group, there was no clinical or laboratory evidence of hyperandrogenemia and the participants had regular menstrual cycles (intermenstrual intervals between 28 and 32 days but with no more than 4 days' variation from cycle to cycle). In addition, they had not been submitted to treatment for menstrual disturbances or infertility at any time.

All subjects included in the study were in good health and, for at least 3 months prior to the study, were off any medication known to affect carbohydrate or sex hormone metabolism. All subjects had performed moderate physical activity (not participating in strenuous physical activities) and had been on a balanced diet (isocaloric) for at least 6 months prior to the study.

The study protocol was approved by the local ethics board and informed consent was obtained from all participants.

Protocol

On the first day, the metabolic study was performed in the Outpatient Section of Endocrinology of the 1st Department of Internal Medicine of the Laiko University Hospital in Athens. After a 10hr overnight fasting, the subjects rested for 30 min in the supine position and blood samples were collected (time 0). Subsequently, 75 gr of glucose was given per os and blood samples were obtained every 30 min for 120 min. On the second day, the hemodynamic study was performed in the Vascular Laboratory, Department of Clinical Therapeutics of the Alexandra University Hospital, in a quiet, temperature-controlled (21-23 °C) room following an over-

night 10hr fast. Firstly, plethysmography was performed and, subsequently, IMT measurement was carried out. Finally, endothelium-dependent dilatation by flow mediated dilatation (FMD) as well as endothelium-independent nitrate-induced dilatation (NID), at the brachial artery, were determined.

Weight, height and waist and hip circumferences were measured. Waist circumference was obtained as the smallest circumference at the level of the umbilicus. Hip circumference was obtained as the widest circumference at the level of the buttocks. BMI and waist-to-hip ratio (WHR) were calculated using appropriate methodologies.

Blood pressure, both Systolic (SBP) and Diastolic (DBP), were measured by a mercury sphygmomanometer with the subject in a sitting position, after a rest of at least 5 min.

The degree of hirsutism was estimated by the Ferriman Gallway scale.²⁰ The evaluations were conducted within the follicular phase of the menstrual cycle in control women and at any time in the PCOS women, who were chronically anovulatory. Chronic anovulation was assessed as oligomenorrhea, i.e. less than 8 cycles per year. In the amenorrhic women, recent ovulation was excluded by progesterone measurement (<5 nmol/l).

A family and a personal history were also recorded.

Hemodynamic studies

Both functional and structural arterial properties were assessed by non-invasive, reproducible methods, namely: flow mediated dilatation (FMD) at the brachial artery, forearm blood flow (FBF) reactive hyperemia (RH) by venous occlusion plethysmography and carotid intima-media thickness (IMT).²¹⁻²³

Plethysmography

The subjects were placed in a supine position with their right arm supported at the elbow and wrist by foam blocks, in a room with consistent temperature. All participants underwent measurement of forearm blood flow (FBF) at baseline and during reactive hyperemia (RH). FBF during RH was measured every 15 sec for three min.

Strain gauge venous occlusion plethysmography, a well known and highly accurate method for the study of small arterioles,^{24,25} was used to study FBF, as previously described.²⁶ Forearm blood flow studies were performed using a strain gauge plethysmograph (model EC5R, Hokanson DE). Mercury-filled silastic strain gauges of appropriate size for each subject were placed 5 cm below the antecubital crease, while a rapid cuff inflator (model E20, Hokanson DE) and an air source (AG101 air source) inflated a vascular cuff to 50mmHg, which was used to occlude venous outflow from the extremity at the level of the upper arm. FBF was calculated as relative percent change of limb volume (mL/min per 100 mL). RH was produced by inflating a wrist cuff to suprasystolic pressure for four minutes. It is known that such duration of arterial occlusion produces close to maximal vasodilation of the blood vessels and maximal peak RH FBF.²⁷

Peak RH FBF corresponded to the maximal RH blood flow that was observed after arterial occlusion release. Time to peak RH FBF was registered as previously described.²⁸ Duration of hyperemia was defined as the time required for hyperemic flow to return to baseline, after peak RH FBF.

Carotid intima-media thickness (IMT)

IMT was measured using B-mode ultrasound imaging (7.0MHz, linear array transducer, Acuson 128XP, Mountain View, California). Scanning included left and right carotid arteries, as previously described.^{29,30} The carotid artery image was focused in the far wall and 3 segments were identified on each side: the distal 1.0 cm of the common carotid proximal to the bifurcation, the carotid bulb and the proximal 1.0 cm of the internal carotid artery. These three measurements of IMT were averaged for each side and, subsequently, the mean value of right and left side (combined) IMT was calculated. The same operator performed IMT measurements for each patient in order to avoid inter-observer variability.

Flow-mediated dilatation

FMD was measured in all subjects non-invasively by B-Mode high-resolution ultrasound imaging (Acuson 128xp; CA, USA).¹⁴ This method has been described previously.^{31,32} Each patient was taken into

a quiet, temperature-controlled room at 20–25° C. After resting in a supine position for 15 min, a 7.0 MHz linear array transducer was used to obtain measurements from the right brachial artery, 1 cm above the elbow joint. Diameter (measured in mm) of the artery was measured at end-diastole, using electronic calipers, by two observers, who were unaware of the study phase. After the resting measurement, a cuff fitted 8 cm distal to the brachial artery and near the wrist was inflated at 250–300 mmHg, altering arterial flow for 4 min. It was then deflated, with subsequent increase of arterial flow (reactive hyperemia). The brachial artery was scanned continuously for 90 sec after cuff deflation, and the vessel's maximal diameter at the same point with resting measurement was defined again (diameter during reactive hyperemia). FMD was calculated as the percent change of the artery's diameter (endothelium-dependent vasodilatation); hyperemia refers to the percent increase of flow. The inter- and intra-observer variability for brachial diameter measurements in our laboratory is 0.1 ± 0.12 and 0.08 ± 0.19 mm, respectively, while FMD variability measured on two separate days was $1.1 \pm 1\%$. Ten minutes after the last scan, a second resting scan was recorded. Afterwards, 0.4mg glyceryl trinitrate was administered sublingually, and 4 min later a last scan was performed in order to measure endothelium independent vasodilatation or nitrate-induced dilatation (NID) to exclude a vascular smooth muscle cell injury.

Assay methods

Blood samples were collected from all patients and healthy controls between 08:00 and 10:00 after an overnight fast. They were centrifuged immediately and serum was stored at -20°C until assayed. The samples were assayed within 12 months of their collection.

All measurements were performed at the Chemwell analyzer (Awareness, Palm City, Florida, USA), unless otherwise stated. Plasma glucose (Glu) was determined by the glucose oxidase color method (Glucose LR, GOD-PAP; Linear Chemicals, Barcelona, Spain). Total cholesterol (TC) was determined by the Enzymatic Cobas Mira method (Cholesterol LR, CHOD-PAP; Linear chemicals). HDL-

Cholesterol (HDL) was assessed enzymatically using a direct method (HDL-Cholesterol, DIRECT, LINEAR CHEMICALS, Barcelona, Spain). Triglycerides (TRIG) were measured using an enzymatic colorimetric method based on hydrolysis of plasma triglyceride to glycerol and free fatty acids by lipoprotein lipase (Triglycerides MR, Linear Chemicals). LDL-Cholesterol (LDL) was calculated by the Friedewald equation. Serum free fatty acids (FFA) were determined using a colorimetric method utilising ACS-ACOD-MEHA reactions (NEFA C, Enzymatic color test, WAKO). Plasma uric acid (UA) concentration was determined by an enzymatic colorimetric method using uricase and peroxidase (Uric Acid LR, URICASE-PAP, Linear Chemicals). Insulin (INS) was measured by a solid phase enzyme amplified sensitivity immunoassay (INS-EASIA; Biosource, Nivelles, Belgium, Europe SA); the intra-assay coefficients of variation (CV) values were 5.3 and 3.0% and the inter-assay values were 9.5 and 4.5% for high and low values, respectively. Within-run CV was 2.7% or less for FFA and under 3% for HDL. Glu and INS concentrations were determined additionally during the OGTT (times 30, 60, 90, 120 min).

Total testosterone (TT) was measured by ELISA (Testosterone Enzyme Immunoassay Test Kit, LI7603; Linear Chemicals). Androstenedione ($\Delta 4A$) was measured by RIA (Active Androstenedione Coated tube RIA kit DSL 3800 (Diagnostic Systems Laboratories, Inc., Webster, TX). Sex hormone binding globulin (SHBG) serum levels were measured by ELISA (SHBG ELISA, MX 520 11; IBL, Hamburg, Germany). Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) were measured using the LHsp and FSH IRMA kits from Biosource Technologies, Inc., Europe S.A. The intra- and inter-assay coefficients of variance for low and high levels, respectively, were: for TT, 5.0 and 6.4 % and 4.4 and 8.4%; for $\Delta 4A$, 5.6 and 2.8%, 9.8 and 7.0%; for SHBG, 3.0 and 5.3% and 7.2 and 8.4%; for LH, 6.5 and 8.8% and 3.5 and 4.5%; for FSH, 2.7 and 5.3% and 1.6 and 3.6%, respectively.

Insulin resistance

Insulin resistance was estimated by the quantitative insulin sensitivity check index (QUICKI) and

Matsuda index.

QUICKI = $1/[\log(\text{fasting insulin}) + \log(\text{fasting glucose})]$.³³

Matsuda index (MATSUDA) was estimated using the following formula:
10000/square root of [(fasting glucose x fasting insulin) x (mean glucose x mean insulin during OGTT)].³⁴

Hyperandrogenemia

The Free Androgen Index (FAI) was estimated using the following formula:

FAI = $[\text{TT (ng/dL)}/\text{SHBG (nmol/L)}] \times 100 (\%)$.³⁵

Statistical analysis

Results are reported as mean values \pm standard error (S.E.). Statistical significance in the results were accepted at a p-value <0.05 . Normal distribution of continuous variables was assessed by applying the non-parametric Kolmogorov–Smirnov test. An independent-sample, two-tailed t-test was used for comparisons between the PCOS women and the control group. The Mann–Whitney U test was performed for variables which were not normally distributed. Correlations between variables were evaluated in the PCOS group only by Pearson's coefficient except for variables not normally distributed, which were evaluated by Spearman's coefficient. Relations between categorical variables were estimated by the chi-square test. Multiple regression analysis was performed in the whole population studied in order to evaluate which variable from PCOS presence (1=PCOS, 0=control), BMI, QUICKI predict hemodynamic the parameters studied.

RESULTS

Women with PCOS had a higher grade of hirsutism severity than the control group ($P < 0.001$). The two groups did not differ in smoking habits ($P = 0.40$), positive family history of type 2 diabetes ($P = 0.17$) or cardiovascular disease ($P = 0.46$) (Table 1).

Hormonal and metabolic parameters are shown in Table 2. All subjects studied exhibited normal glucose tolerance. The PCOS group consisted of 11 (40.7%) normal weight, 9 (33.3%) overweight and 7 (26%) obese women. The control group consisted of 15 (55.6%) normal-weight, 6 (22.2%) overweight and 6 (22.2%) obese women. No difference was observed in BMI distribution between the two groups ($p = 0.52$). Women with PCOS had higher levels of TT ($P < 0.001$), $\Delta 4A$ ($P < 0.001$), LH ($P = 0.03$), higher values of FAI index ($P < 0.001$) and LH-to-FSH ratio ($P = 0.01$) and lower values of QUICKI ($P = 0.04$) and MATSUDA indices ($P = 0.006$).

FMD values were lower in the PCOS group compared to controls ($P < 0.001$), but no difference was observed in NID and in SBP and DBP values (Table 3).

In plethysmography parameters, the only difference was observed in the time to peak reactive hyperemia, which was longer in PCOS women ($P = 0.02$) (Table 3).

No difference was observed in combined IMT values between the studied groups (Table 3).

In women with PCOS, FMD was positively related to QUICKI index ($r = 0.40$, $p = 0.04$) and ne-

Table 1. Clinical features, smoking habits and family history of type 2 diabetes and cardiovascular disease in women with PCOS and in controls.

	PCOS [N/total N (%)]	Controls [N/total N (%)]	P for comparison between PCOS and control group
Ferriman Gallway scale >6	24/27 (88.9)	1/27 (3.7)	<0.001
Smokers	9/27 (34.6)	13/27 (48.1)	0.40
Positive family history for type 2 diabetes	14/26 (53.8)	9/27 (33.3)	0.17
Positive family history for cardiovascular disease	3/27 (11.1)	5/27 (19.2)	0.46

Table 2. Anthropometric, hormonal and metabolic parameters in PCOS and control women. Mean value (\bar{x}), standard error (SE), P-value

	PCOS (N=27) $\bar{x} \pm SE$	Controls (N=27) $\bar{x} \pm SE$	P for comparison between PCOS and control groups
Age (yrs)	25.41±0.80	27.33±0.83	0.10
BMI (kg/m ²)	27.42±1.12	25.05±1.19	0.16
WHR	0.78±0.01	0.75±0.01	0.13
Total T (nmol/L)	2.97±0.19	1.38±0.09	<0.001
FAI*	339.08±48.90	106.43±11.46	<0.001
SHBG (nmol/l)	34.57±3.49	43.25±2.21	0.08
FSH (U/L)	4.98±0.33 (4.98±0.33)	5.40±0.52 (5.40±0.52)	0.48
LH (U/L)	8.47±0.97	5.84±0.66	0.03
Androstenedione (nmol/L)	10.85±0.76	5.37±0.38	<0.001
LH/FSH ratio	1.83±0.21	1.14±0.14	0.01
Glucose (mmol/L)	83.11±2.19 (4.61±0.12)	81.00±2.87 (4.5±0.16)	0.46
Insulin (basal) (pmol/L)*	94.00±16.10	62.40±7.00	0.06
Total cholesterol (mmol/L)	4.52±0.25	4.23±0.18	0.33
HDL cholesterol (mmol/L)	1.30±0.06	1.31±0.05	0.92
LDL cholesterol (mmol/L)	2.98±0.35	2.66±0.21	0.44
Triglycerides (mmol/L)	0.98±0.15	0.77±0.08	0.23
FFA (mmol/L)	0.02±0.001	0.02±0.01	0.39
Uric acid (μmol/L)	273.00±11.00	250.00±11.00	0.15
QUICKI index	0.342±0.005	0.359±0.005	0.04
MATSUDA index	3.83±0.37	6.77±0.91	0.006

*Not normally distributed. Mann Whitney test used

BMI: body mass index; WHR: waist-to-hip Ratio; T: testosterone; FAI: free androgen index; SHBG: sex hormone binding globulin; FSH: follicle stimulating hormone; LH: luteinizing hormone.

Table 3. Hemodynamic, functional and structural parameters in PCOS and control women. Mean value (\bar{x}), standard error (SE), P-value

	PCOS (N=27) $\bar{x} \pm SE$	Controls (N=27) $\bar{x} \pm SE$	P for comparison between PCOS and control group
Systolic blood pressure (mmHg)	114.81±2.85	111.66±2.32	0.38
Diastolic blood pressure (mmHg)	73.89±2.25	71.30±1.70	0.36
Baseline artery diameter (mm)	3.05±0.06	3.17±0.09	0.27
Flow mediated dilatation (%)	3.84±0.74	9.83±0.97	<0.001
Nitrate induced dilatation (%)	16.59±1.84	16.64±2.05	0.98
Forearm blood flow at rest (ml/100ml/min)	4.85±0.40	5.25±0.41	0.49
Peak reactive hyperemia (ml/100ml/min)	9.43±0.71	11.25±1.11	0.18
Time to peak reactive hyperemia (sec)*	20.63±4.67	10.38±5.11	0.02
Duration of reactive hyperemia (sec)	93.75±12.18	90.58±10.47	0.84
Intima media thickness [combined value (mm)]	0.49±0.01	0.51±0.02	0.19

* Not normally distributed. Mann Whitney test used.

gatively to UA ($r=-0.54$, $p=0.004$), TRIG ($r=-0.50$, $p=0.02$), basal INS ($r=-0.40$, $p=0.04$), and FFA ($r=-0.92$, $p=0.008$). FBF at rest was positively related to peak RH ($r=0.64$, $p=0.001$). Time to peak RH was negatively related to SHBG ($r=-0.40$, $p=0.04$). Peak RH was positively related to FBF at rest and to duration of RH ($r=0.42$, $p=0.03$). Duration of RH was positively related to peak RH and FAI index ($r=0.40$, $p=0.05$) and negatively to SHBG ($r=-0.43$, $p=0.03$). Combined IMT was positively related to TC ($r=0.62$, $p=0.001$) and LDL ($r=0.80$, $p<0.001$) (Table 4).

In multiple regression analysis it was revealed that between PCOS presence, BMI and QUICKI, FMD values were predicted by PCOS presence ($b=-0.561$, $p<0.001$, $R^2=0.29$, $F=8.06$) and IMT was predicted by BMI ($b=0.005$, $p=0.04$, $R^2=0.10$, $F=2.94$).

Table 4. Correlation of hemodynamic, functional and structural vascular parameters studied in PCOS group.

Variable	R	P
Flow mediated dilatation		
QUICKI	0.40	0.04
Uric acid	-0.54	0.004
Triglycerides	-0.50	0.02
Insulin	-0.40	0.04
Free fatty acids	-0.92	0.008
Forearm blood flow at rest		
Peak reactive hyperemia	0.64	0.001
Time to peak reactive hyperemia		
SHBG	-0.40	0.04
Peak reactive hyperemia		
Forearm blood flow at rest	0.64	0.001
Duration of reactive hyperemia	0.42	0.03
Duration of reactive hyperemia		
Peak reactive hyperemia	0.42	0.03
SHBG	-0.43	0.03
FAI index	0.40	0.05
Intima media thickness combined		
Total cholesterol	0.62	0.001
LDL cholesterol	0.80	<0.001

DISCUSSION

In the present study we examined both microvascular and macrovascular function, as well as arterial structural characteristics in women with PCOS and in controls using non-invasive methodologies. We showed that endothelial function in the macrocirculation, assessed by FMD, was impaired. Furthermore, we found evidence of early impairment of microcirculation, as assessed by the time required for reactive hyperemia to reach peak value. Both alterations apparently precede the development of structural arterial wall deterioration, since, at this time, no difference in IMT values between the PCOS women and controls was detected.

Controversial data exist regarding the presence of endothelial dysfunction and arterial structure impairment in women with PCOS, certain studies reporting no impairment,^{8-12,36,37} and others showing significant impairment.⁹⁻¹⁷ Differences concerning cardiovascular risk factors among the various populations studied might explain these discrepancies. It should be mentioned that in the present study, subjects with PCOS did not differ in classical parameters of the metabolic syndrome (except for insulin-resistance) from the control women, suggesting the presence of an "early" stage of dysmetabolic syndrome in the PCOS group. This fact may explain the absence of increased IMT in the PCOS women studied in contrast to the previously reported data by Orio et al.¹² Vrionidou et al.¹⁷ found increased IMT values in PCOS women but in the presence of a higher incidence of family history for type 2 diabetes, and higher glycemia, lipidemia and WHR compared to the control group on the latter study. In the present study, the findings about carotid arteries are in accordance with data of Talbott et al.,³⁶ who observed a difference between PCOS subjects and controls in carotid IMT in older women (aged ~45 years) but not in younger women. These findings suggest that measurable vascular abnormalities in PCOS women are possibly developed by middle age. Additionally, Meyer et al.³⁷ did not find increased IMT in young overweight PCOS, despite higher HDL and triglycerides levels compared with the control group. In our study, IMT values in PCOS women were positively related to lipidemia, a well-

documented risk factor for increased IMT values;³⁸ however, lipidemia did not differ between PCOS population and controls.

Endothelial dysfunction seems to be a well recognised trait in the PCOS syndrome⁹⁻¹⁶ in both micro- and macrocirculation, although contradictory results have also been reported.⁸ Paradisi et al.⁹ were the first to recognise the presence of endothelial dysfunction in PCOS microcirculation by the use of invasive leg plethysmography, but differences in HDL and triglycerides plasma levels between PCOS and control women in this study may have accounted for these findings. We have also previously reported that endothelial dysfunction, as assessed by both increased ET-1 plasma levels and FMD, is present in PCOS,^{10,14} a finding which was confirmed by Orio et al.¹² However, brachial artery diameter differences between controls and PCOS in the latter study could reflect an overt impairment of the arterial structure. Tarkun et al.¹³ and Kravariti et al.¹⁶ found altered FMD and NID, suggesting a global vascular injury and not only endothelial dysfunction. Lakhani et al.¹⁵ found impaired microvascular function by the use of invasive venous occlusive plethysmography and intra-arterial application of acetylcholine, but the differences in BMI between PCOS and control women could have played a role in the observed endothelial dysfunction. Finally, Kelly et al.¹¹ found impaired microvascular function by the use of an invasive method.

The novelty of the present study was that the micro- and macrocirculation were simultaneously assessed by a non-invasive methodology. FMD at the brachial artery was lower in the presence of PCOS, although peak forearm blood flow reactive hyperemia was comparable in the two groups, implying the presence of earlier injury in conduit arteries, independent of an overt microcirculation injury.³⁹ On the other hand, both the early phase of reactive hyperemia, assessed by the maximal reactive hyperemia blood flow, and the late phase of reactive hyperemia, assessed by the duration of reactive hyperemia, were preserved. Early microvascular dysfunction was suggested by the delayed vasoreactivity response, assessed by the time required for reactive hyperemia forearm blood flow to peak.

This finding might be explained by previous observations of Westerbacka et al. in insulin resistance states⁴⁰ in which peripheral vasoreactivity was preserved despite delayed response.

A positive relationship between endothelial dysfunction and insulin resistance indices was found. It is possible that insulin-resistant individuals exhibit resistance to both the metabolic and vascular actions of insulin. Phosphoinositide 3 kinase (PI-3K), a key intracellular signalling step of insulin action, has been shown by *in vivo* studies to be defective in the insulin resistant state as well as in PCOS.⁴¹ The signalling pathways by which insulin mediates glucose uptake and NO production converge at the PI-3K/Akt.^{42,43} Consequently, disruption of this pathway may lead to impaired glucose utilization and endothelial dysfunction. Furthermore, hyperinsulinemia drives endothelin 1 (ET-1) production by endothelial cells via the PI-3K pathway, further impairing endothelial function by competing with NO; ET-1 has been shown to inhibit insulin signalling via the same pathway.^{44,45} Furthermore, ET-1 levels have been found increased in PCOS subjects.^{10,12,14} These findings support the hypothesis that in PCOS women, the abnormal vasculature status could be due to impaired insulin vasodilator functions, mediated by PI3-K.⁴⁶

Moreover, the hyperandrogenemia index was related to duration of reactive hyperemia in women with PCOS. There are data which support a role of hyperandrogenemia in vascular reactivity in PCOS,⁹ but the mode of action of androgens remains unknown. Androgen receptors are known to exist on the vessel wall, and a direct effect of androgens in the vasculature cannot be excluded.⁴⁷ Alternatively, androgens may act synergistically with insulin resistance,⁴⁸ inflammatory cytokines⁴⁹ or vasoconstrictive peptides¹⁰ on endothelial function. Androgens may promote monocyte adhesion to endothelial cells a proatherogenic effect mediated, at least in part, by an increased endothelial cell-surface expression of VCAM-1,⁵⁰ which has been found elevated in PCOS subjects.⁵¹

PCOS presence predicted FMD values independently of BMI or insulin resistance indices (hyperandrogenemia and menstrual irregularities), imply-

ing an additive role of hyperandrogenemia in endothelial dysfunction. The complex correlation between vascular parameters, insulin resistance and hyperandrogenemia suggests that these factors are interrelated and interplay possibly with other unknown factors affecting the vascular bed of a young population of PCOS women who do not carry an increased load of cardiovascular risk factors.

Among the limitations of this study is the fact that in order to examine vascular status without the interference of other cardiovascular risk factors, we have possibly underestimated endothelial dysfunction and arterial structural impairment, since women with PCOS carry, in general, several metabolic aberrations. Another limitation of this study is the small number of patients studied given that it is difficult to recruit women with PCOS with only insulin resistance and no other cardiovascular risk factors.

In conclusion, this study demonstrates that young PCOS women at the early dysmetabolic stage exhibit macrovascular and to a lower degree microvascular dysfunction, without evidence of arterial structural impairment, when assessed by non-invasive methods. Early endothelial dysfunction appears to be a more sensitive and/or earlier index than structural arterial changes and therefore might be more appropriate to evaluate this parameter in follow-up studies, which implicate interventions of lifestyle changes or drug therapy.

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