CLINICAL STUDY

Metformin administration improves endothelial function in women with polycystic ovary syndrome

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Abstract

Objective: The aim of this study was to investigate the endothelial status in young women with polycystic ovary syndrome (PCOS), using a simple and easily reproducible hemodynamic method combined with a biological marker and to evaluate the effect of metformin treatment on these parameters. **Design:** Descriptive clinical trial.

Methods: Forty young women, 20 with PCOS and 20 normal women of similar age and body mass index were studied. Metformin (1700 mg daily) was administered for 6 months to the PCOS group. The endothelium status and the metabolic and hormonal profile were studied in both groups, as well as after metformin, by flow-mediated dilatation (FMD) on the brachial artery and by measurements of plasma endothelin-1 (ET-1) levels.

Results: FMD was impaired in the PCOS group when compared with controls ($3.24 \pm 0.71\%$ vs $8.81 \pm 1.07\%$ respectively, $P < 0.0001$), but this difference normalized after metformin treatment (PCOS_{post-metformin} vs controls: 8.17 ± 1.26 vs $8.81 \pm 1.07\%$, $P = 0.70$) since the values significantly improved after metformin treatment (PCOS_{pre-metformin} vs PCOS_{post-metformin}: 3.24 ± 0.71 vs $8.17 \pm 1.26\%$, $P = 0.003$). ET-1 levels were significantly higher in the PCOS women compared with the control group (7.23 ± 0.50 vs 4.99 ± 0.69 fmol/l, $P = 0.01$), they improved significantly after metformin treatment (PCOS_{pre-metformin} vs PCOS_{post-metformin}: 7.23 ± 0.50 vs 3.57 ± 0.60 fmol/l, $P < 0.0001$) and their difference compared with the control group was reversed (PCOS_{post-metformin} vs controls: 3.57 ± 0.60 vs 4.99 ± 0.69 fmol/l, $P = 0.13$). Metformin administration improved hyperandrogenemia. However, in this study, mathematical methods used to assess insulin resistance failed to show any detected alteration after treatment with metformin.

Conclusions: PCOS women were found to exhibit endothelial dysfunction compared with controls, which was reversed 6 months after metformin administration.

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Introduction

Polycystic ovary syndrome (PCOS), affecting 4–7% of women of reproductive age (1, 2), bears a risk for development of cardiovascular disease and type 2 diabetes (T2D) (3–9). The major surrogate markers for cardiovascular risk factors identified in PCOS are coronary calcifications assessed by electron beam computed tomography (EBCT), carotid intima media thickness by ultrasound and arterial stiffness by recording pulse wave velocity (PWV) across the brachial artery (10–12). Furthermore, endothelial dysfunction has been investigated by different methods in women with PCOS with contradictory results (13–16).

Endothelial dysfunction is defined as a change in concentration of the chemical messengers (like endothelin-1 (ET-1)) produced by endothelium and/or

as a blunting of the nitric oxide (NO)-dependent vasodilator response to hyperemia (17). Additionally, the disruption of the balance between the endothelial production of protective vasoactive molecules such as NO and the generation of deleterious substances, such as ET-1, may participate in the mechanisms of the atherosclerotic process (17, 18). Insulin resistance may be linked to endothelial dysfunction by a number of mechanisms, including disturbances of subcellular signaling pathways common to both insulin action and NO production or other potential unifying links such as oxidant stress, ET-1, the renin–angiotensin system and the secretion of hormones and cytokines by adipose tissue (18).

Different approaches have been used to assess endothelial integrity (13–16). Paradisi *et al.* (14) found that obese, insulin-resistant women with PCOS exhibit

impaired endothelial function (measured by an invasive hemodynamic method), which has been correlated with insulin resistance and testosterone plasma levels. Mather *et al.* (13) failed to demonstrate endothelial abnormality assessed by flow-mediated dilatation (FMD) on the brachial artery in women with PCOS, compared with normal women. These hemodynamic methods are considered to largely reflect NO production/release (19). In our previous studies, it was demonstrated that ET-1 plasma levels were elevated and FMD was impaired in different populations of PCOS (15, 20). Orio *et al.* (16) confirmed the relationship of vascular damage with ET-1 plasma levels. Insulin resistance has been shown to have a negative correlation with endothelial function; a therapeutic intervention with insulin-sensitizer has been proven beneficial (21, 22). Therefore, the aim of this study was to evaluate the effect of metformin administration on endothelial status in PCOS.

Subjects and methods

Subjects

The study consisted of 40 women, 20 women with PCOS, all recruited from the Outpatient Department of Endocrinology of the Laiko University Hospital in Athens between 2001 and 2003, and 20 normal women (doctors and students) who volunteered. The study protocol was approved by the local ethics board and informed consent was obtained from all participants. All the participants 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. Twenty women (mean age: 24.95 ± 1.11 years; body mass index (BMI): $28.37 \pm 1.59 \text{ kg/m}^2$ (range: $16.46\text{--}39.80 \text{ kg/m}^2$)) with the diagnosis of PCOS were recruited. Their diagnosis was based on the presence of irregular menstrual cycles (eight or fewer menses per year), elevated plasma levels of testosterone and clinical symptoms of hyperandrogenism, according to the National Institute of Child Health and Human Development conference. Non-classical congenital adrenal hyperplasia, androgen-secreting neoplasm, hyperprolactinemia and thyroid disease were excluded by appropriate tests in the PCOS women. Twenty normal, healthy, women served as the control group (mean age: 26.00 ± 0.90 years; BMI: $26.59 \pm 1.30 \text{ kg/m}^2$ (range: $20.33\text{--}40.15 \text{ kg/m}^2$)). In this group, there was no hyperandrogenemia or evidence of hyperandrogenism (hirsutism, acne or alopecia) on physical examination; they had regular menstrual cycles (intermenstrual intervals between 25 and 35 days but with no more than 4 days' variation from cycle to cycle), and had not sought treatment for menstrual disturbances or infertility at any time.

Protocol

The metabolic study and the follow up were performed on day 1 in the Outpatient Department of Endocrinology, First Department of Internal Medicine in Laiko University Hospital in Athens. After a 12 h overnight fasting period, on the morning of day 1 the subjects were rested for 30 min in the supine position, and blood samples were collected (time 0). Subsequently, an oral glucose tolerance test (OGTT) with 75g glucose load was performed at 30 min intervals (times 30, 60, 90, 120).

The hemodynamic study was performed on day 2 in the Vascular Laboratory, Department of Clinical Therapeutics in Alexandra University Hospital. All studies were performed in a quiet, temperature-controlled room after an overnight 14 h fast. Endothelium-dependent FMD of the brachial artery, a well-known method for measurement of endothelial function and also endothelium-independent (nitrate-mediated) vasodilatation (NMD) was obtained in all subjects.

All subjects who were current smokers were requested to reduce the number of cigarettes for a 1 week period and not to smoke 2 days before the hemodynamic study, in order to be included in the study.

Weight, height and waist and hip circumferences were measured. BMI was calculated by the formula: $\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$. Waist-to-hip ratio (WHR) was calculated by the formula: $\text{WHR} = \text{waist circumference (cm)} / \text{hip circumference (cm)}$. 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.

Blood pressure was measured by a mercury sphygmomanometer with the subject in a sitting position, after a rest of at least 5 min. The average of three measurements was obtained.

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. In the amenorrheic women, recent ovulation was excluded by progesterone measurement ($< 5 \text{ nmol/l}$). Blood samples were collected between 0800 and 1000 h after an overnight fast.

After baseline studies, metformin was administrated in women with PCOS for 6 months. At the end of this period they underwent repeat studies of hemodynamic, hormonal and metabolic parameters under the same conditions. The PCOS women were closely followed up for the entire period of the study.

Hemodynamic studies

FMD was measured in all subjects non-invasively by B-Mode high-resolution ultrasound imaging (Acuson 128xp; CA, USA) (23). This method has been described previously (24, 25). 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 at a specific anatomical point. 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. Then it was deflated, with subsequent increase of arterial flow (reactive hyperemia).

The brachial artery was scanned continuously for 90 s 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, 0.4 mg glyceryl trinitrate was administered sublingually, and 4 min later a last scan was performed in order to measure endothelium independent vasodilation or nitrate-mediated dilation (NMD) to exclude a vascular smooth muscle cell injury.

Assay methods

Blood samples were centrifuged immediately, and serum was stored at -20°C until assayed. The samples were assayed within 3–9 months of their collection.

All measurements were performed at the Chemwell analyzer (Palm City, Florida, USA), unless otherwise stated.

Plasma glucose 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). Insulin was measured by a solid phase enzyme amplified sensitivity immunoassay (INS-EASIA; Biosource, Nivelles, Belgium, Europe SA). The intra-assay coefficient 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. Total testosterone was measured by ELISA (Testosterone Enzyme Immunoassay Test Kit, LI7603; Linear Chemicals). The expected normal values for testosterone (as in the kit insert) are 0.2–0.8 ng/ml for premenopausal females in the follicular phase. Sex hormone-binding globulin (SHBG) serum levels were measured by ELISA (SHBG ELISA, MX 520 11; IBL, Hamburg, Germany). The expected values for women for SHBG are 15–120 nmol/l.

Hormonal and biochemical parameters

Serum levels of total testosterone (nmol/l), SHBG (nmol/l), serum fasting insulin (INS, pmol/l), serum fasting glucose (GLU, mmol/l) and TC (mmol/l) were measured on day 1, at baseline (time 0), before metformin, as well as after 6 months metformin/administration. Glucose and insulin concentrations were determined additionally during the OGTT (times 30, 60, 90, 120 min) on day 1 before metformin as well as 6 months after metformin administration.

ET-1 measurement

Blood samples (5 ml) for ET-1 determination were collected into tubes containing EDTA. They were immediately centrifuged at -4°C for 20 min at 3000 rpm, and serum stored at -40°C . ET-1 (fmol/ml) was measured at day 1, at baseline, before metformin as well as 6 months after metformin administration by an endothelin (1–21) Test Kit from Biomedica, Wien, Germany, which is an enzyme immunoassay that makes use of a highly specific monoclonal detection antibody for endothelin (1–21). The intra- and interassay CV values for ET-1 were 4.5 and 4.4% and 6.9 and 7.6%, for high and low values respectively.

Insulin resistance estimation

Insulin resistance was estimated by the quantitative insulin sensitivity check index (QUICKI) and Matsuda index. The glucose and insulin response to glucose were also assessed by calculating the area under the curve (AUC) during OGTT performance for glucose (AUCGLU) and insulin (AUCINS), using the trapezoidal rule. QUICKI is defined as: $\text{QUICKI} = 1/[\log(\text{fasting insulin}) + \log(\text{fasting glucose})]$ (26). The Matsuda index is obtained using the following formula: $\text{Matsuda index} = 10\,000/\text{square root of } [(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin during OGTT})]$ (27).

Metformin protocol

Twenty women with PCOS received a dose of 1700 mg metformin daily for 6 months (Lipha Sante; Aron Medica Division, Lyon, France). Initially, metformin was administered in incremental doses (i.e. (850/2) mg, 850 mg, (850 + 850/2) mg, 1700 mg) every 7 days until the final dose of 1700 mg daily. All women were urged to maintain the same diet followed prior to treatment and were checked monthly. No severe side effects were reported during the study. 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. After 6 months of treatment, the haemodynamic, hormonal and metabolic

studies were repeated (i.e. 6 months after metformin administration measurements).

Statistical analysis

Results are reported as mean values $\pm$ S.E. Statistical analysis was 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 t -test was used for comparisons between PCOS women and the control group and a paired t -test was applied to evaluate changes between measurements at baseline and after the 6 month treatment period. Mann–Whitney U and Wilcoxon tests were performed for variables which were not normally distributed. Correlations between variables were evaluated by Pearson's coefficient except for variables not normally distributed, which were evaluated by Spearman's coefficient. Multiple regression analysis was applied to estimate which of testosterone, BMI, SHBG, Matsuda index and ET-1 as independent variables best predict the value of FMD as the dependent variable, and which of testosterone, BMI, SHBG, Matsuda index and FMD as the independent variables best predict the value of ET-1 as dependent variable. A chi-square test was applied to compare the smoking habit and the diabetes family history for the two groups.

Analysis was performed using SPSS (Statistical Package for the Social Sciences, version 11.01; SPSS, Inc., Chicago, IL, USA) for Windows XP (Microsoft Corp.).

Results

Baseline study

Demographic profile The PCOS women and the controls did not differ in age, BMI or WHR (Table 1). Seven and ten women were currently light smokers (i.e. no more than 10 cigarettes per day for the last 5 years)

in the PCOS and control groups respectively ($P = 0.33$). Ten and nine women had a positive family history of T2D in the PCOS and control group respectively ($P = 0.75$).

Hemodynamic profile The studied groups did not differ in baseline artery diameter. Before treatment, the PCOS group differed in FMD compared with the control group with a statistically significant difference (PCOS_{pre-metformin}: $3.24 \pm 0.71\%$ vs controls: $8.81 \pm 1.07\%$, $P < 0.0001$, observed power = 0.99) (Fig. 1). No difference was observed in NMD between the control group and the PCOS pre-treatment (PCOS_{pre-metformin}: $13.34 \pm 1.67\%$; controls: $15.67 \pm 1.68\%$). The PCOS group before treatment had higher values than controls in systolic blood pressure (SBP) (PCOS_{pre-metformin}: 114.35 ± 2.80 mmHg; controls: 110.24 ± 2.69 mmHg, $P = 0.29$) and in diastolic blood pressure (DBP) (PCOS_{pre-metformin}: 76.25 ± 2.34 mmHg; controls: 69.75 ± 2.19 mmHg, $P = 0.05$) but the difference was statistically significant only in DBP (Table 2).

ET-1 levels ET-1 levels were significantly higher in the PCOS women before treatment compared with the control group (Table 2).

Hormonal profile The PCOS group before treatment differed statistically significantly from the control group in testosterone levels but not in SHBG levels (Table 1).

Metabolic profile Three PCOS women exhibited impaired glucose tolerance and they could have introduced bias into the study. However, their inclusion in the study did not change our results in the measured variables. The PCOS group before treatment did not differ from the control group in GLU, INS and TC (Table 1). The PCOS group before treatment differed from the control group with statistically significant

Table 1 Anthropometric characteristics and hormonal and metabolic profiles of PCOS (P) patients pre-metformin (M) and post-metformin and of normal control women (C). Data as means $\pm$ S.E.

Variable	P values					
	P pre-M (n = 20)	P post-M (n = 20)	C (n = 20)	P pre-M vs C	P pre-M vs P post-M	P post-M vs C
Age (years)	24.95 $\pm$ 1.11	24.95 $\pm$ 1.11	26.00 $\pm$ 0.90	0.046	—	0.46
BMI (kg/m ²)	28.37 $\pm$ 1.59	28.85 $\pm$ 1.69	26.59 $\pm$ 1.30	0.39	0.23	0.29
WHR	0.77 $\pm$ 0.018	0.79 $\pm$ 0.021	0.74 $\pm$ 0.016	0.28	0.46	0.10
Testosterone (nmol/l)	3.17 $\pm$ 0.22	2.21 $\pm$ 0.26	1.40 $\pm$ 0.10	< 0.0001	0.01	0.008
SHBG (nmol/l)	30.28 $\pm$ 3.25	34.05 $\pm$ 3.82	38.93 $\pm$ 3.16	0.06	0.35	0.33
Glucose (mmol/l)	4.62 $\pm$ 0.14	4.81 $\pm$ 0.13	4.47 $\pm$ 0.13	0.47	0.31	0.08
INS (pmol/l)	114.9 $\pm$ 22.64	104.9 $\pm$ 20.48	66.46 $\pm$ 9.02	0.06	0.77	0.06
TC (mmol/l)	4.64 $\pm$ 0.32	3.96 $\pm$ 0.24	4.23 $\pm$ 0.15	0.28	0.01	0.36
QUICKI	0.335 $\pm$ 0.007	0.332 $\pm$ 0.007	0.357 $\pm$ 0.006	0.04	0.74	0.02
AUCGLU	941.2 $\pm$ 32.86	987.4 $\pm$ 30.59	767.6 $\pm$ 38.92	< 0.0001	0.41	< 0.0001
AUCINS	88615 $\pm$ 13284	84270 $\pm$ 11495	54197 $\pm$ 7266	0.02	0.51	0.03
Matsuda index	3.52 $\pm$ 0.43	2.83 $\pm$ 0.27	5.97 $\pm$ 0.64	0.002	0.15	< 0.0001

$P < 0.05$ statistically significant.

Figure 1 Endothelial function measured by FMD in women with PCOS. * $P < 0.05$ vs control; § $P < 0.05$ vs PCOS after metformin.

differences in AUCGLU, AUCINS, QUICKI and Matsuda index (Table 1).

Post-metformin study

Demographic profile BMI and WHR remained unchanged after metformin therapy (Table 1). The women with PCOS did not change smoking habits.

Hemodynamic profile FMD was statistically higher after treatment (PCOS_{pre-metformin}: $3.24 \pm 0.71\%$ vs PCOS_{post-metformin}: $8.17 \pm 1.26\%$, $P = 0.003$, observed power = 0.94) (Fig. 1). Additionally, the PCOS group after treatment did not differ from the controls group in FMD ($P = 0.70$). No difference was observed in NMD between the PCOS group before and after treatment and between PCOS after treatment and controls (PCOS_{pre-metformin}: $13.34 \pm 1.67\%$; PCOS_{post-metformin}: $15.88 \pm 3.18\%$; controls: $15.67 \pm 1.68\%$). In the PCOS group, the values of both SBP and DBP were decreased after metformin but without reaching a statistically significant difference (PCOS after treatment: SBP: 112.36 ± 3.42 mmHg ($P = 0.53$); DBP: 72.89 ± 2.91

mmHg ($P = 0.34$)). Consequently no statistical significant difference was found in BP values between the PCOS group after treatment and controls (Table 2).

ET-1 levels In the PCOS group, ET-1 levels were statistically lower after treatment, and the PCOS group after treatment did not differ from the control group in ET-1 plasma levels (Table 2).

Hormonal profile Testosterone plasma levels were statistically lower after treatment, while SHBG was not altered; the PCOS group after treatment differed in testosterone levels compared with the control group, with a statistically significant difference, but not in SHBG levels (Table 1).

Metabolic profile GLU and INS were not altered by metformin treatment, but a statistically significant decrease was observed in TC; the PCOS group after treatment did not differ from the control group in GLU, INS or TC (Table 1). AUCGLU, AUCINS, QUICKI and the Matsuda index remained unchanged after metformin treatment; the PCOS group after treatment differed from the control group with statistically significant differences in AUCGLU, AUCINS, QUICKI and the Matsuda index (Table 1).

Correlations FMD was negatively related to testosterone levels ($r = -0.338$, $P = 0.03$), AUCGLU ($r = -0.391$, $P = 0.01$), INS ($r = -0.34$, $P = 0.03$) and SBP ($r = -0.455$, $P = 0.004$), and positively related to the Matsuda index ($r = 0.431$, $P = 0.008$); ET-1 plasma levels were positively related to testosterone levels (Table 3). Additionally, FMD was negatively related to ET-1 levels ($r = -0.239$) but this relationship was not statistically significant ($P = 0.13$). In multiple regression analysis testosterone and the Matsuda index were the independent predictors of FMD values ($P = 0.05$, $b = -0.040$ and $P = 0.01$, $b = 0.702$ respectively) between BMI, SHBG and ET-1. On the other hand, testosterone was the only independent predictor of ET-1 values ($P = 0.09$, $b = 0.023$) between BMI, SHBG, the Matsuda index and FMD.

Table 2 Brachial artery response expressed as percent dilatation from baseline, ET-1 plasma levels, SBP and DBP values. PCOS (P), metformin (M), controls (C). Data as means $\pm$ S.E.

Variable	P values					
	P pre-M (n=20)	P post-M (n=20)	C (n=20)	P pre-M vs C	P pre-M vs P post-M	P post-M vs C
Baseline artery diameter (mm)	3.15 $\pm$ 0.06	3.19 $\pm$ 0.05	3.24 $\pm$ 0.10	0.48	0.46	0.68
FMD (%)	3.24 $\pm$ 0.71	8.17 $\pm$ 1.26	8.81 $\pm$ 1.07	<0.0001	0.003	0.70
NMD (%)	13.34 $\pm$ 1.67	15.88 $\pm$ 3.18	15.67 $\pm$ 1.68	0.34	0.30	0.85
ET-1 (fmol/ml)	7.23 $\pm$ 0.50	3.57 $\pm$ 0.60	4.99 $\pm$ 0.69	0.01	<0.0001	0.13
SBP (mmHg)	114.35 $\pm$ 2.80	112.36 $\pm$ 3.42	110.24 $\pm$ 2.69	0.29	0.53	0.62
DBP (mmHg)	76.25 $\pm$ 2.34	72.89 $\pm$ 2.91	69.75 $\pm$ 2.19	0.05	0.34	0.39

$P < 0.05$ statistically significant.

Table 3 Results from correlation analyses for FMD and ET-1 for the population studied shown as Pearson's coefficient for variables normally distributed and Spearman's coefficient for variables not normally distributed.

Variable	<i>r</i>	<i>P</i>
FMD		
Testosterone	−0.38	0.03
AUCGLU	−0.391	0.01
INS	−0.34	0.03
SBP	−0.455	0.004
Matsuda index	0.431	0.008
ET-1		
Testosterone	0.307	0.05

Discussion

In the present study is demonstrated impaired endothelial function, assessed by hemodynamic (FMD) and biochemical methods (ET-1) in PCOS women, which was normalized to control levels after metformin therapy. In women with PCOS, impaired macrovascular and microvascular function has been found with laborious methods like EBCT, ultrasounds equipped with specific systems, or by PWV, and myography by muscle biopsy (10–12), which have technical or psychological costs. It is interesting to note that in this study the findings of endothelial dysfunction by FMD are in agreement with the data of invasive techniques (14, 21). Additionally, the highly reproducible and non-invasive technique, FMD, is combined with ET-1 plasma levels, after excluding a smooth muscle cells vascular injury. This combination may be more suitable for a global approach and follow-up of endothelial status of young women with PCOS. FMD values were lower and ET-1 plasma levels were higher in PCOS women compared with controls of similar age and BMI. Increased ET-1 plasma levels have been described in patients with atherosclerotic lesions (28, 29) and insulin-resistant populations (18, 30–35). Furthermore, impaired endothelium-dependent vasodilatation has been reported to be associated with insulin-resistant states (36, 37).

There are studies which provide evidence of inter-correlation of insulin, NO and ET-1 signaling pathways in insulin-resistant individuals. This could be applied to PCOS women, since they also present impaired insulin action (38–43).

Endothelial function improved after metformin administration, despite no detected alteration in insulin resistance indices. The Matsuda index, however, was found to be an independent predictor of FMD, and correlated positively with FMD, supporting the interaction of these two parameters, in the studied group. Interestingly, it has been shown that insulin resistance may be difficult to assess accurately by other methods or calculations when the basal insulin levels are in the normal or high-normal range in PCOS women (44, 45). Alternatively, it is possible that the beneficial effect after

metformin on endothelial function could also be mediated by other mechanisms.

DBP also improved; this finding could be of clinical significance for women with PCOS (46). Additionally, in this study, BMI cannot be included in the parameters affecting the endothelial function, since BMI changes were not detected after treatment.

Hyperandrogenemia improved after metformin therapy, and a positive correlation with endothelial dysfunction was demonstrated with both FMD and ET-1 plasma levels. The mechanism by which hyperandrogenemia might affect vascular reactivity is still unknown. Androgen receptors are known to exist on the vessel wall, and a direct effect of androgens in the vasculature cannot be excluded (47, 48). Alternatively, androgens may act synergistically with insulin resistance (49), inflammatory cytokines (50) or angio-constrictive peptides (15) on endothelial function.

A drop in TC levels after metformin therapy could be due to the decreased androgens levels, as hyperandrogenism has been shown to affect lipoproteins and lipids independently of insulin levels and body weight (9, 51).

The limitations of our study should also be taken into consideration: this is a non-placebo, non-randomized therapeutic trial, and insulin resistance was not assessed by the gold standard method of a euglycemic clamp.

In conclusion, this study demonstrates the presence of abnormal endothelial status, functional and biochemical, in PCOS women, which is normalized 6 months after metformin administration. This therapeutic intervention may modify in the early stages of the atherosclerotic process, and could be of clinical importance in this high-risk group of young women.

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