Effect of the insulin sensitizers metformin and pioglitazone on endothelial function in young women with polycystic ovary syndrome: a prospective randomized study

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Objective: To compare the effect of two different insulin sensitizers, metformin and pioglitazone, on endothelial function in women with polycystic ovary syndrome (PCOS).
Design: Prospective randomized study.
Setting: University Hospital endocrinology outpatient clinic.
Patient(s): Young women with PCOS (aged 23.3 ± 4.9 years).
Intervention(s): Patients were assigned randomly to no treatment (n = 14), metformin 850 mg two times per day (n = 15), and pioglitazone 30 mg daily (n = 14) for 6 months. Healthy age- and body mass index–matched women served as controls (n = 14).
Main Outcome Measure(s): Brachial artery flow-mediated dilation was studied at baseline and 6 months.
Result(s): Women with PCOS had higher insulin resistance and hyperandrogenism indices and lower flow-mediated dilation compared with controls. The three groups of women with PCOS did not differ at baseline. No differences were observed at follow-up in women who received no treatment. Metformin and pioglitazone improved flow-mediated dilation to a similar extent, restoring it to normal values at 6 months. Both insulin sensitizers induced favorable changes in insulin resistance and hyperandrogenism indices in women with PCOS. Independent predictors of flow-mediated dilation improvement at 6 months were treatment with insulin sensitizers and reduction in insulin resistance.
Conclusion(s): In young women with PCOS, treatment with metformin or pioglitazone for 6 months induces a similar beneficial effect on endothelial function; this may be partially attributed to an improvement in insulin resistance. Further research is needed to investigate whether treatment with insulin sensitizers in women with PCOS also reduces cardiovascular risk. (Fertil Steril® 2011;95:203–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, endothelial function, metformin, pioglitazone, atherosclerosis

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder, characterized by the presence of chronic oligo-ovulation or anovulation, hyperandrogenism, and polycystic ovaries (1). Women with PCOS demonstrate a high incidence of insulin resistance and cardiovascular risk factors such as dyslipidemia, hypertension, obesity, and type 2 diabetes (2–4), indicating increased risk for future cardiovascular disease events. Furthermore, it is well established that in women with PCOS subclinical atherosclerosis is evident at a young age (5–12). Evidence is accumulating that long-term cardiovascular morbidity may be increased in women with PCOS, although the data are limited and nonconclusive (13, 14).

Endothelial dysfunction occurs early in the process of atherosclerosis (15), and its presence, as assessed by reduced brachial artery flow-mediated dilation, has been associated with increased risk for future cardiovascular events (16, 17). Previous studies in young women with PCOS demonstrated endothelial dysfunction compared with age-matched healthy women (5, 8–10, 12), and this impairment in vascular endothelium has been related mainly to insulin resistance and/or hyperandrogenism (5, 9, 10).

Insulin sensitizers, such as metformin and pioglitazone (a thiazolidinedione), have been used in addition to lifestyle changes for the treatment of PCOS and have shown beneficial effects in improving ovulation (18–20), hyperandrogenism, and insulin resistance (19–22). Thus, it can be speculated that these medications also may improve endothelial function in women with PCOS and possibly decrease cardiovascular risk. Conflicting results have been reported regarding the effects of metformin on flow-mediated dilation in women with PCOS (21–23), whereas rosiglitazone,

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another thiazolidinedione, has recently been shown to improve flow-mediated dilation in women with PCOS (24). The effects of pioglitazone on flow-mediated dilation have not been reported in women with PCOS. The aim of this study was to compare the effects of metformin and pioglitazone administration for 6 months on endothelial function in young women with PCOS.

MATERIALS AND METHODS

Study Population

Forty-five young Greek women (aged ≤35 years) with established PCOS were recruited consecutively from the endocrine outpatients' clinic of the Department of Endocrinology at the University Hospital of Ioannina. Polycystic ovary syndrome was defined when at least two of the following three features were present (after the exclusion of other etiologies such as congenital adrenal hyperplasia, hyperprolactinemia, thyroid disease, or Cushing's syndrome): oligo-ovulation or anovulation (fewer than six menstrual cycles in the preceding year), hyperandrogenism, and polycystic ovaries (1). Hyperandrogenism was defined by the clinical presentation of hirsutism (Ferriman-Gallwey score >8), acne, or male pattern alopecia, and/or elevated androgen levels. Polycystic ovaries were defined by the transvaginal ultrasound appearance of 12 or more follicles in each ovary measuring 2 to 9 mm in diameter and/or ovarian volume >10 mL (1). Fourteen healthy age- and body mass index (BMI)–matched regularly menstruating women (menstrual cycles between 21 and 35 days) were recruited from the hospital and university staff and served as controls.

Women with PCOS and control women were excluded from the study if they had [1] prior treatment (in the past 6 months) known to affect vascular endothelial function (vitamins, antioxidants, cardiovascular medications); [2] prior treatment (in the past 6 months) with oral contraceptives, antiandrogens, glucocorticoids, or infertility medications; [3] history of cardiovascular disease or diabetes; or [4] excessive alcohol use (more than two drinks a day). Smoking history was recorded, but it was not an exclusion criterion.

Study Design

This was a prospective, randomized, open-label study evaluating the effect of 6-month treatment with metformin or pioglitazone on flow-mediated dilation in women with PCOS. Secondary outcomes were changes in other clinical, hormonal, and metabolic variables. Women with PCOS eligible for the study who consented to participate were assigned randomly following a computer-generated list of randomization into three groups (n = 15 in each group): no treatment in group 1, metformin 850 mg two times per day in group 2, and pioglitazone 30 mg once per day in group 3. During the recruitment visit in the clinic, simple advice on healthy diet and regular exercise had been given to all women. Control women were used for comparison with women with PCOS at baseline and also at 6 months to assess whether restoration to normality was achieved.

Forty-three women with PCOS completed the study; one woman in group 1 (no treatment) and one in group 3 (pioglitazone) were lost to follow-up and were not included in data analysis. All other women in groups 2 and 3 received the assigned per-group treatment as checked by tablet counts at the end of follow-up. Both medications were well tolerated, and no adverse events were recorded. Women with PCOS did not receive any other medications, and smoking status did not change during the 6 months. In all three groups, essentially no lifestyle changes were reported.

Blood samples were drawn both at baseline and at the end of the 6-month follow-up, after an overnight fast, in the early follicular phase (days 3–5) of a spontaneous or progestin-induced menstrual cycle. Levels of glucose, insulin, sex hormone-binding globulin (SHBG), T, and lipids were measured. All women underwent a standard oral glucose tolerance test at baseline and 6 months. Endothelial function was assessed the next morning. Blood sampling and assessment of endothelial function also were performed in control women at baseline.

The study was approved by the Institutional Review Board of the University Hospital of Ioannina, Greece, and all study participants provided written informed consent. The study complies with the Declaration of Helsinki.

Biochemical Assays

Plasma glucose and lipids were determined with use of standard methods. Insulin was measured by microparticle enzyme immunoassay on an AXSYM immunoanalyzer (Abbott Laboratories, Abbott Park, IL). For glucose and insulin determinations during the oral glucose tolerance test, samples from each subject were analyzed together in the same assay to avoid interassay variability. Total T was determined by chemiluminescent microparticle immunoassay on an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratories). Sex hormone-binding globulin was measured by chemiluminescent immunometric method (IMMULITE 2000 immunoanalyzer; Diagnostic Products Co., Los Angeles, CA). The ratio of [T (nmol/L) × 100]/SHBG (nmol/L) was used to calculate the free androgen index (FAI).

Insulin Sensitivity/Resistance Indices

The fasting glucose-to-insulin ratio was calculated as fasting glucose (mg/dL)/fasting insulin (μIU/mL). Composite whole-body insulin sensitivity index (ISI) was calculated according to the following formula: 10,000/square root of [(Fasting glucose, mmol/L) (Fasting insulin, pmol/L) (Mean insulin during oral glucose tolerance test, pmol/L) (Mean glucose during oral glucose tolerance test, mmol/L)]. The insulin area under the curve (I_{AUC120}), an index of insulin resistance, was evaluated with use of the trapezoidal rule.

Assessment of Endothelial Function

Brachial artery flow-mediated dilation and nitrate-mediated dilation were assessed as previously described (10, 25, 26). All studies were performed at the Michaelidion Cardiac Center, University of Ioannina, Ioannina, Greece, by the same operator, who was unaware of the hormonal status and treatment of the women. Optimal imaging of the right brachial artery was obtained with use of an Echo-Doppler ultrasound system (Ultrasound ATL; HDI 5000, Bothell, WA) and a 5-12 MHz transducer. Images were recorded on super-VHS videotape (VCR AG-MD835; Panasonic, Osaka, Japan) for off-line analysis. Brachial artery diameter was measured by a blinded reader at end-diastole. Images were acquired at baseline; during hand hyperemia, that is, 90 seconds after deflation of a wrist cuff inflated to suprasystolic pressure for 5 minutes for measurement of flow-mediated dilation; and at 4 minutes after 400 μg of sublingual glyceryl trinitrate for measurement of nitrate-mediated dilation. Flow-mediated dilation was calculated as the percent increase in arterial diameter during hyperemia compared with the diameter at rest, and hyperemic flow was measured as the peak flow at 15 seconds after cuff release with use of continuous-wave Doppler. Published internal repeatability data (10) demonstrate the accuracy and repeatability of these parameters.

Statistical Analysis

Continuous data are presented as mean ± SD. All variables were found to be normally distributed. Independent samples *t*-test and χ^2 test were used to compare continuous and categorical variables respectively between controls and women with PCOS. One-way ANOVA, with the Bonferroni or Tamhane test for post-hoc comparisons, was used to compare baseline differences among the three groups of women with PCOS. To assess changes from baseline at follow-up within each group, the paired Student's *t*-test was used. To compare variables between controls and each of the three PCOS groups (at baseline and 6 months), the independent samples *t*-test was used. Repeated-measures ANOVA was implemented to compare changes in flow-mediated dilation and all other studied variables at 6-month follow-up between the groups. Power calculations were performed on the basis of our previous data in PCOS (10) and the degree of anticipated change in flow-mediated dilation. For a 100% change in flow-mediated dilation with 90% power, 12 subjects were required in each group for two-sided $P=.01$.

The relationship between flow-mediated dilation changes at follow-up and the studied demographic, clinical, metabolic, and hormonal characteristics (at baseline and absolute changes at follow-up) was assessed with bivariate correlation analysis. Variables for which correlation with flow-mediated dilation changes achieved near statistical significance ($P<.1$) were entered into a stepwise regression model to assess the magnitude of their individual effects on flow-mediated dilation. *P* values were always two-sided, and

a value of $<.05$ was considered significant. The SPSS statistical software package (version 15.0 for Windows; SPSS, Inc. Chicago, IL) was used.

RESULTS

Baseline Comparisons; Controls Versus Women With PCOS and Among the Three Groups of Women With PCOS

The clinical, metabolic, hormonal, and vascular parameters of the women with PCOS and control women are shown in Table 1. Women with PCOS and control women differed significantly in glucose-to-insulin ratio, ISI, I_{AUC120}, Ferriman-Gallwey score, total T and SHBG levels, FAI, systolic blood pressure (SBP), flow-mediated dilation, and nitrate-mediated dilation ($P<.05$ for all) (Table 1). Table 2 summarizes clinical, metabolic, hormonal, and vascular measurements at baseline and 6 months in all groups of women with PCOS. The three groups did not differ in any studied parameters at baseline (P not significant for all). There were significant differences between each of the three groups of women with PCOS and controls, similar to the differences observed between the whole group of women with PCOS and controls (shown in Table 1).

Effects of Metformin and Pioglitazone

In group 1, no significant differences were observed at 6 months compared with baseline (Table 2), and indices of insulin resistance, hyperandrogenism, and vascular function at 6 months remained significantly different compared with controls ($P<.05$ vs. controls). In group 2, a significant improvement was observed at 6 months in fasting insulin, glucose-to-insulin ratio, ISI, I_{AUC120}, low-density lipoprotein cholesterol (LDL-c), total T, FAI, SBP, diastolic blood pressure, and flow-mediated dilation ($P<.05$ for all) (Table 2). Flow-mediated dilation was restored to control values at 6 months (Fig. 1), and ISI, I_{AUC120}, total T, and FAI remained significantly different compared with controls ($P<.05$ vs. controls). In group 3, an improvement was observed at 6 months in fasting insulin, glucose-to-insulin ratio, ISI, I_{AUC120}, high-density lipoprotein cholesterol (HDL-c), Ferriman-Gallwey score, total T, FAI, and flow-mediated dilation ($P<.05$ for all). An increase in body weight, BMI, waist circumference, and total cholesterol ($P<.05$ for all) also was found (Table 2). A trend for an increase in nitrate-mediated dilation ($P=.07$) and in LDL-c ($P=.06$) was observed with pioglitazone. Flow-mediated dilation was restored to control values with pioglitazone (Fig. 1), as well as nitrate-mediated dilation (P not significant vs. controls for both), whereas Ferriman-Gallwey score, total T, and FAI at 6 months remained significantly different compared with controls ($P<.05$ vs. controls).

Comparison Between Metformin and Pioglitazone

With use of repeated-measures ANOVA (Table 2), it was found that treatment with metformin or pioglitazone induced a similar improvement in flow-mediated dilation and hyperandrogenism indices (Ferriman-Gallwey score, total T, and FAI) (P not significant between groups). There were significant differences between the pioglitazone and metformin groups ($P<.05$) in the treatment-induced changes in body weight, BMI, total cholesterol levels (all

TABLE 1

Clinical, metabolic, hormonal, and vascular characteristics in control women and women with PCOS at baseline.

Characteristic	Control women (n = 14)	Women with PCOS (n = 43)	*P* value
Age, y (range)	25.0 ± 5.3 (18–34)	23.3 ± 4.9 (18–35)	.3
Smokers, n (%)	5 (35.7)	11 (25.6)	.5
Family history of CAD, n (%)	4 (28.6)	7 (16.3)	.4
Weight (kg)	71.6 ± 6.8	75.9 ± 14.6	.1
BMI (kg/m^2)	26.8 ± 2.7	28.7 ± 5.5	.1
Waist circumference (cm)	83.6 ± 7.5	85.9 ± 12.5	.4
Waist-to-hip ratio	0.81 ± 0.03	0.79 ± 0.05	.07
Glucose (mg/dL)	89 ± 8	88 ± 7	.7
Insulin (μIU/mL)	8.9 ± 6.7	12.2 ± 5.0	.05
Glucose-to-insulin ratio ($mg \cdot mL \cdot dL^{-1} \cdot \mu IU^{-1}$)	11.7 ± 7.7	6.8 ± 2.9	.04*
ISI ($L^2 \cdot mmol^{-1} \cdot pmol^{-1}$)	20.2 ± 8.1	9.6 ± 3.9	<.001*
I_{AUC120} ($\mu IU \cdot min/mL$)	3,680 ± 2,014	9,428 ± 4,747	<.001*
TC (mg/dL)	183 ± 48	183 ± 26	.9
LDL-c (mg/dL)	117 ± 37	119 ± 22	.8
HDL-c (mg/dL)	50 ± 13	44 ± 9	.2
TRG (mg/dL)	82 ± 29	97 ± 38	.2
Ferriman-Gallwey score	4.1 ± 3.5	10.1 ± 3.1	<.001*
Total T (ng/dL)	39.1 ± 15.4	93.3 ± 24.5	<.001*
SHBG (nmol/L)	50.7 ± 15.2	32.1 ± 17.6	.001*
FAI (%)	2.9 ± 1.2	13.5 ± 8.3	<.001*
SBP (mm Hg)	111 ± 7	118 ± 10	.03*
DBP (mm Hg)	69 ± 5	73 ± 9	.2*
Baseline EDD (mm)	3.09 ± 0.22	3.17 ± 0.32	.4*
Flow-mediated dilation (%)	9.36 ± 2.78	4.28 ± 2.35	<.001*
Nitrate-mediated dilation (%)	23.31 ± 4.26	18.31 ± 4.36	.001*

Note: CAD = coronary artery disease; DBP = diastolic blood pressure; EDD = end-diastolic diameter; TC = total cholesterol; TRG = triglycerides.
* Significant differences between the groups.

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TABLE 2

Clinical, metabolic, hormonal, and vascular characteristics in the three groups of women with PCOS at baseline and 6 months.

Characteristic	Group 1, no treatment (n = 14)			Group 2, metformin (n = 15)			Group 3, pioglitazone (n = 14)			*P* value, groups 1 vs. 2[a]	*P* value, groups 1 vs. 3[a]	*P* value, groups 2 vs. 3[a]
	Baseline	After 6 mo	*P* value	Baseline	After 6 mo	*P* value	Baseline	After 6 mo	*P* value			
Age (y)	24.3 ± 6.0	—	—	22.2 ± 3.6	—	—	23.6 ± 5.1	—	—			—
Weight (kg)	75.4 ± 14.1	74.8 ± 15.5	.5	78.8 ± 16.2	78.5 ± 16.1	.8	73.5 ± 13.8	76.9 ± 14.3	.02	.8	.01*	.04*
BMI (kg/m2)	28.3 ± 4.9	28.1 ± 5.5	.5	29.4 ± 6.5	29.3 ± 6.5	.8	28.5 ± 5.4	29.8 ± 5.7	.02	.8	.01*	.04*
Waist (cm)	84.6 ± 12.1	85.4 ± 13.7	.3	87.6 ± 12.7	88.4 ± 13.1	.6	85.3 ± 13.5	88.1 ± 12.3	.04	.9	.1	.3
Waist-to-hip ratio	0.79 ± 0.05	0.80 ± 0.06	.1	0.79 ± 0.05	0.81 ± 0.06	.08	0.80 ± 0.06	0.79 ± 0.05	.4	.5	.1	.06
Glucose (mg/dL)	89 ± 8	89 ± 5	.9	90 ± 5	87 ± 6	.3	86 ± 7	88 ± 6	.3	.4	.5	.1
Insulin (μIU/mL)	11.8 ± 4.2	11.7 ± 4.8	.9	12.7 ± 5.3	9.9 ± 4.6	.01*	12.1 ± 5.8	7.5 ± 4.3	.002	.09	.01*	.3
Glucose-to-insulin ratio ($mg \cdot mL \cdot dL^{-1} \cdot \mu IU^{-1}$)	6.8 ± 2.7	7.1 ± 2.7	.7	6.4 ± 2.1	8.0 ± 2.6	.02*	7.2 ± 4.0	13.3 ± 9.0	.02	.2	.02*	.05
ISI ($L^2 \cdot mmol^{-1} \cdot pmol^{-1}$)	9.8 ± 4.3	11.2 ± 5.5	.1	9.0 ± 3.2	11.4 ± 3.6	.01*	10.1 ± 4.5	17.3 ± 8.4	< .001	.3	< .001*	.002*
IAUC120 (μIU/mL·min)	8,877 ± 3,612	8,108 ± 3,549	.5	10,161 ± 5,755	7,616 ± 3,492	.01*	9,134 ± 4,724	5,186 ± 2,170	.002	.2	.04*	.3
TC (mg/dL)	177 ± 22	180 ± 16	.6	181 ± 21	174 ± 25	.2	191 ± 33	212 ± 47	.009	.2	.04*	.004*
LDL-c (mg/dL)	113 ± 18	115 ± 15	.7	119 ± 20	109 ± 27	.05*	127 ± 27	140 ± 42	.06	.07	.1	.006*
HDL-c (mg/dL)	42 ± 8	43 ± 7	.6	43 ± 10	45 ± 9	.4	47 ± 9	55 ± 10	.009*	.7	.04*	.08
TRG (mg/dL)	108 ± 49	109 ± 58	.9	97 ± 37	103 ± 39	.1	86 ± 24	84 ± 23	.9	.5	.8	.4
Ferriman-Gallwey score	10.4 ± 4.6	9.9 ± 3.9	.2	9.8 ± 2.2	8.6 ± 2.8	.05	10.0 ± 2.0	8.4 ± 2.0	.006*	.3	.07	.6
T (ng/dL)	92.5 ± 26.3	99.1 ± 19.6	.5	98.6 ± 24.5	76.3 ± 20.1	.02*	88.4 ± 23.2	73.4 ± 31.6	.02*	.03*	.06	.5
SHBG (nmol/L)	32.0 ± 17.3	30.5 ± 13.5	.6	31.7 ± 18.5	33.3 ± 14.8	.4	32.7 ± 18.8	36.6 ± 18.3	.2	.4	.2	.5
FAI (%)	13.4 ± 9.0	14.6 ± 9.0	.6	14.4 ± 8.8	9.3 ± 5.4	.009*	12.7 ± 7.6	8.1 ± 4.6	.01*	.03*	.04*	.8
SBP (mm Hg)	116 ± 8	113 ± 7	.1	121 ± 8	114 ± 13	.03*	116 ± 13	114 ± 9	.4	.3	.7	.2
DBP (mm Hg)	70 ± 5	71 ± 6	.9	74 ± 8	71 ± 6	.01*	73 ± 12	74 ± 8	.9	.08	.9	.9
Baseline EDD (mm)	3.16 ± 0.30	3.10 ± 0.27	.4	3.18 ± 0.35	3.13 ± 0.37	.5	3.18 ± 0.34	3.19 ± 0.44	.8	.9	.4	.5
Flow-mediated dilation (%)	4.36 ± 1.82	4.17 ± 2.21	.8	4.07 ± 2.66	9.33 ± 2.20	< .001*	4.43 ± 2.61	9.32 ± 3.14	< .001*	< .001*	< .001*	.8
Nitrate-mediated dilation (%)	18.37 ± 2.90	17.96 ± 2.70	.7	18.22 ± 4.95	19.39 ± 4.76	.4	18.22 ± 5.32	21.33 ± 3.81	.07	.3	.07	.3

Note: DBP = diastolic blood pressure; EDD = end-diastolic diameter; TC = total cholesterol; TRG = triglycerides. Data are shown as mean ± SD unless otherwise stated.

* Significant differences between the groups.

[a] Repeated-measures ANOVA.

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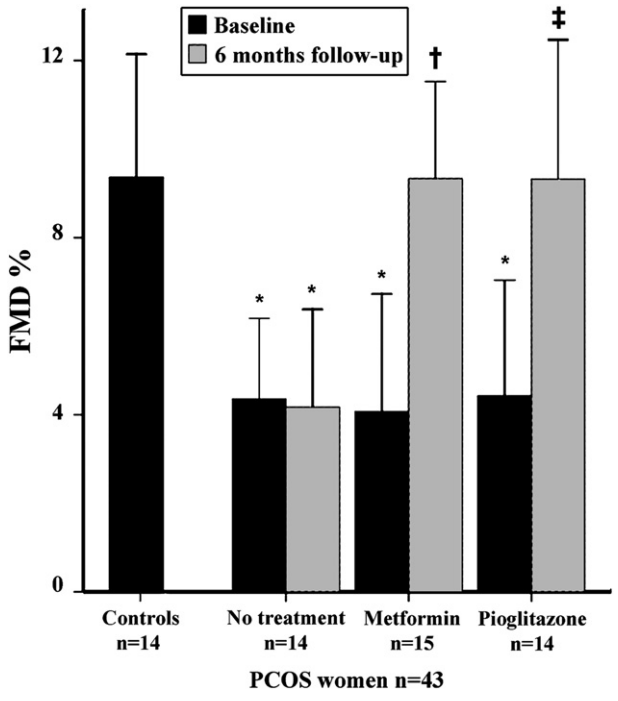
FIGURE 1

Flow-mediated dilation (FMD) in the control women and in the three groups of women with PCOS at baseline and at 6-month follow-up. *$P<.001$ versus controls. †$P<.001$ versus baseline in metformin group. ‡$P<.001$ versus baseline in pioglitazone group.



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increased with pioglitazone), LDL-c levels (increase with pioglitazone vs. decrease with metformin), ISI (greater increase with pioglitazone), and also glucose-to-insulin ratio ($P=.05$). A trend for a difference between groups also was observed for changes in waist-to-hip ratio (increase with metformin vs. decrease with pioglitazone, $P=.06$) and in HDL (greater increase with pioglitazone, $P=.08$). Compared with group 1, treatment with metformin (group 2) induced a significant increase in flow-mediated dilation ($P<.0001$), in FAI, and in total T levels ($P<.05$ for both). Compared with group 1, treatment with pioglitazone (group 3) induced a significant increase in flow-mediated dilation, body weight, BMI, glucose-to-insulin ratio, ISI, and total and HDL-c levels, and a significant decrease in I_{AUC120} and FAI ($P<.05$ for all).

Independent Predictors of Treatment-induced Changes in Flow-mediated Dilation

The parameters associated with flow-mediated dilation changes at 6 months in women with PCOS (n = 43) are shown in Table 3. Stepwise regression analysis revealed that the independent predictors of flow-mediated dilation improvement at 6 months were treatment with an insulin sensitizer and reduction in I_{AUC120} at follow-up (r^2 0.48, $P<.0001$). Treatment with an insulin sensitizer accounted for approximately 39% of the variance of flow-mediated dilation changes (slope 4.28, $P<.0001$, 95% confidence interval: 2.30, 6.27), whereas reduction in I_{AUC120} at 6 months accounted for another 9% (slope $-3.1\cdot10^{-4}$, $P=.015$, 95% confidence interval: -0.001, $-6.4\cdot10^{-5}$).

TABLE 3

Correlations between absolute changes in brachial artery flow-mediated dilation at 6-month follow-up and relevant clinical, metabolic, and hormonal parameters in women with PCOS.

	Women with PCOS (n = 43)	
Parameter	***r***	***P* value**
Treatment with any insulin sensitizer	0.644	<.0005
I_{AUC120} (μIU/mL·min)	0.308	.05
Changes in fasting insulin (μIU/mL) at follow-up	−0.325	.034
Changes in ISI ($L^2\cdot mmoL^{-1}\cdot pmoL^{-1}$) at follow-up	0.437	.004
Changes in I_{AUC120} (μIU/mL·min) at follow-up	−0.472	.002
Changes in Ferriman-Gallwey score at follow-up	−0.350	.022

Note: Only significant correlations at the $P<.1$ level are shown.

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DISCUSSION

The present study demonstrates impairment of endothelium-dependent vasodilation in young women with PCOS compared with healthy age- and BMI-matched controls. Metformin and pioglitazone treatment for 6 months in women with PCOS resulted in a similar improvement in flow-mediated dilation that was restored to normal values. The two insulin sensitizers had differential effects on obesity measures and lipid profile, and amelioration of insulin resistance indices was probably greater with pioglitazone. Hyperandrogenism indices were improved similarly with both medications. Treatment with insulin sensitizers and reduction in insulin resistance at follow-up were found to be independent predictors of the flow-mediated dilation increase in women with PCOS.

Our study provides clear evidence of early impairment of endothelial function in young women with PCOS who are not dyslipidemic or hypertensive compared with age- and BMI-matched controls in accordance with previous data (5, 8–10, 12). Endothelial dysfunction, assessed by reduced flow-mediated dilation, has shown promising results in cardiovascular risk stratification and prognosis (16, 17). Another vascular abnormality observed in this study in women with PCOS was reduced nitrate-mediated dilation, indicating impaired endothelium-independent vasodilation, a finding reported previously (9, 10).

Metformin administration for 6 months in women with PCOS induced a significant increase in flow-mediated dilation that was restored to normal values with a concomitant decrease in insulin resistance and hyperandrogenism indices. A decrease in LDL-c, SBP, and diastolic blood pressure were additional beneficial effects observed with metformin in accordance with previous reports (22, 27). Conflicting evidence regarding the effect of metformin on endothelial function in women with PCOS has been reported. Two studies reported normalization of flow-mediated dilation and improvement of insulin resistance and hyperandrogenism with 6-month metformin treatment in young women with PCOS (21, 22), whereas another study failed to show any benefit of

metformin on endothelial function or hyperandrogenism, despite a significant improvement in insulin resistance (23).

Pioglitazone administration for 6 months in women with PCOS resulted in an increase in flow-mediated dilation that was restored to normal values, as well as nitrate-mediated dilation, and an improvement in insulin resistance and hyperandrogenism indices. In addition, a beneficial effect on HDL-c was seen with pioglitazone, whereas an increase in body weight, BMI, waist circumference, and total and LDL cholesterol also was observed. Similar effects of pioglitazone have been reported previously in patients with type 2 diabetes (26, 28). In women with PCOS, pioglitazone administration has been shown to reduce soluble markers of atherosclerosis (29) and indices of insulin resistance and hyperandrogenism (19, 20). Rosiglitazone, another thiazolidinedione, has been shown to improve endothelial function and hormonal and metabolic parameters in women with PCOS (24, 30).

The results of our study indicate that these two different insulin sensitizers, metformin and pioglitazone, have a similar effect on flow-mediated dilation, similar to previous data comparing metformin and rosiglitazone (24) and despite a slightly greater improvement in insulin resistance indices (ISI and glucose-to-insulin ratio) with pioglitazone. This may be attributed to the differential effects of metformin and pioglitazone on metabolic factors such as obesity measures, blood pressure, and lipids (20, 27) and also to other "pleiotropic" vascular actions exerted by insulin sensitizers such as modification of adipokines' production or vascular inflammation (31, 32). The results of this study further indicate that the independent predictors of the flow-mediated dilation improvement at 6 months were treatment with an insulin sensitizer and improvement in insulin resistance (expressed by decrease in I_{AUC120}). Reduction in insulin resistance with metformin or rosiglitazone treatment has been associated further with an increase in flow-mediated dilation (22, 30).

Insulin sensitizers improved hyperandrogenism indices measured in our study (total T, Ferriman-Gallwey score, FAI); the reduction in Ferriman-Gallwey score with treatment correlated with flow-mediated dilation increase at follow-up (Table 3), but this improvement did not have an independent predictive value for flow-mediated dilation change. In agreement with our results, reduction in FAI with metformin for 6 months could not predict the observed increase in flow-mediated dilation in young normal-weight women with PCOS (22).

Regarding limitations, this was a prospective, randomized, single-center study that assessed endothelial function with use of flow-mediated dilation, and biochemical markers of endothelial function were not included. Approximately 50% of the flow-mediated dilation variance could be explained by our model, suggesting that other factors may play an important role. Total T was measured, and FAI was calculated in this study because free T measurement by equilibrium dialysis is not available in our laboratory. Because our goal was to compare the effect on flow-mediated dilation of the two insulin sensitizers, only simple advice on diet and exercise was given to women with PCOS; thus, minimal lifestyle changes were reported that did not influence our results.

In conclusion, young women with PCOS have impaired endothelial function indicating an increased risk for early onset cardiovascular disease. Treatment with metformin or pioglitazone for 6 months induces a similar beneficial effect on endothelial function that is restored to normal values. This may be attributed partially to an improvement in insulin resistance, and other metabolic and nonmetabolic actions of insulin sensitizers also may be contributing. Long-term studies are needed to investigate whether treatment with insulin sensitizers also may improve cardiovascular prognosis in women with PCOS.

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