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Predictors of Endothelial Dysfunction in Young Women with Polycystic Ovary Syndrome

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Context: Women with polycystic ovary syndrome (PCOS) may be at increased risk for cardiovascular disease. Endothelial dysfunction is an early marker of atherosclerosis.

Objectives: The objectives of this study were to 1) compare endothelial function in young women with PCOS and regularly menstruating control women, and 2) to identify the determinants of endothelial function and investigate its relationship with body mass index in women with PCOS.

Design: This was a cross-sectional study.

Setting: This study was conducted at a tertiary cardiovascular research center.

Patients: Sixty-two young women with PCOS (mean age, 22.7 yr) and 17 control women, matched as a group for age and body mass index, were studied. Twenty-three women with PCOS were lean, 21 were overweight, and 18 were obese.

Main Outcome Measures: Endothelium-dependent and -independent vascular function was assessed by measuring flow-mediated dilation (FMD) and nitrate-mediated dilation in the brachial artery (diameter change during hand hyperemia and after sublingual glyceryl trinitrate administration, respectively).

Results: FMD and nitrate-mediated dilation were significantly lower in PCOS than in control women (reduced by approximately 50 and 25%, respectively; both $P < 0.0005$). Insulin resistance, total testosterone, and total cholesterol were independent predictors of FMD, accounting for 21, 10, and 9% of the variance, respectively ($P < 0.005$ for all). A trend of deterioration in FMD from lean to overweight and obese PCOS women was observed, but differences among groups were not statistically significant.

Conclusions: Women with PCOS have significant endothelial dysfunction at an early age (*i.e.* early 20s), and largely independent of obesity. This suggests that women with PCOS are at increased risk for early onset cardiovascular disease and may gain particular benefit from measures to improve endothelial function. **(*J Clin Endocrinol Metab* 90: 5088–5095, 2005)**

THE POLYCYSTIC OVARY syndrome (PCOS) is the most common endocrinopathy in women, affecting 5–10% of women of reproductive age (1). PCOS is characterized by oligo-/anovulation and hyperandrogenism and is associated with multiple risk factors for cardiovascular disease, such as insulin resistance, central adiposity, dyslipidemia, and hypertension (2). It is now known that all these cardiovascular risk factors are associated with endothelial dysfunction, an early marker of atherosclerosis (3), and that the magnitude of this defect predicts cardiovascular events (4).

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Abbreviations: ANCOVA, Analysis of covariance; BMI, body mass index; DBP, diastolic blood pressure; FAI, free androgen index; F-G, Ferriman-Gallwey; FMD, flow-mediated dilation; G_0, basal level of glucose; GIR, glucose to insulin ratio; GTN, glyceryl trinitrate; HDL, high-density lipoprotein; I_0, basal level of insulin; I_{AUC120}, insulin area under the curve; ISI(composite), composite whole-body insulin sensitivity index; LDL, low-density lipoprotein; NMD, nitrate-mediated dilation; NO, nitric oxide; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TC, total cholesterol; TRG, triglyceride.

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Although data regarding cardiovascular morbidity and mortality in PCOS women are conflicting (5–7), recent studies suggest that the risk of cardiovascular disease is increased in this group of women (6, 8–10). Evidence regarding endothelial function in women with PCOS is contradictory. In obese women with PCOS, Mather *et al.* (11) reported normal endothelial function, whereas Paradisi *et al.* (12) demonstrated endothelial dysfunction and insulin resistance at a vascular level. In younger and nonobese women with PCOS, two recent studies showed impairment of endothelial function and vascular structure (13, 14).

The objectives of our study were 1) to compare endothelial function in young women with PCOS and control women matched as a group for age and body mass index (BMI), and 2) to identify the determinants of endothelial function in women with PCOS and to investigate its relationship with BMI.

Subjects and Methods

Study design

In this cross-sectional study, endothelium-dependent and -independent vascular function was evaluated in young women with PCOS and

in healthy women with normal ovarian function matched as a group for age and BMI (control group).

Sixty-two Greek young women with established PCOS (mean age, 22.7 yr; range, 18–35 yr) were studied. PCOS 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- or amenorrhea (<six menstrual cycles in the preceding year), hyperandrogenism, and polycystic ovaries (15). 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 ultrasound appearance of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or ovarian volume greater than 10 ml. Exclusion criteria for PCOS women included current or previous use (within 6 months) of oral contraceptives, antiandrogens, statins, glucocorticoids, or infertility medications.

The BMI of each subject, calculated as weight (kilograms)/height (meters)^2, was recorded. The presence and extent of hirsutism was quantified using the Ferriman-Gallwey (F-G) score. The minimum waist measurements between the pelvic brim and the costal margin and the maximum hip measurement at the level of the greater trochanters were used to calculate the waist to hip ratio.

The control group consisted of 17 women matched as a group for age and BMI with the PCOS women. Control women had normal ovaries (on transvaginal ultrasound examination), regular menstrual cycles (intermenstrual intervals of 21–35 d), and no signs of hyperandrogenism (hirsutism or acne). All subjects (PCOS and controls) were nonpregnant, nonsmokers, normotensive, and euglycemic. None of the subjects was taking any medications that could influence vascular function.

The study was approved by the Ethics Committee of the Michaelidion Cardiac Center (University of Ioannina, Greece), and all study participants provided written informed consent.

Procedures

Basal levels of glucose (G_0), insulin (I_0), SHBG, testosterone, LH, FSH, and lipids were measured in all subjects, after an overnight fast, in the early follicular phase (d 3–5) of a spontaneous or progestin-induced menstrual cycle. In addition, all subjects underwent a standard oral glucose tolerance test (OGTT). Before and at 30, 60, 90, and 120 min after the ingestion of oral glucose (75 g), blood was sampled for plasma glucose and serum insulin levels. Endothelial function was assessed the next day.

Laboratory investigations

Plasma glucose was determined by the hexokinase method using a glucose analyzer (Olympus 600, Clinical Chemistry Analyser, Olympus Diagnostica GmbH, Hamburg, Germany). Insulin was measured by microparticle enzyme immunoassay on an AXSYM immunoanalyzer (Abbot Laboratory, Abbott Park, IL). For glucose and insulin determinations during the OGTT, samples from each subject were analyzed together in the same assay to avoid interassay variability. Total testosterone and serum gonadotropins (LH and FSH) were determined by chemiluminescent microparticle immunoassay on an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratory). SHBG was measured by chemiluminescent immunometric method (IMMULITE 2000 immunoanalyzer, Diagnostic Products Co., Los Angeles, CA). The ratio of total testosterone × 100/SHBG was used to calculate the free androgen index (FAI) (16). Serum total cholesterol (TC) and triglycerides (TRGs) were determined by enzymatic colorimetric assay (Olympus, AU560; Diagnostica, Hamburg, Germany). High-density lipoprotein (HDL) cholesterol was determined enzymatically in the supernatant after dextran-magnesium-induced precipitation of other lipoproteins. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula: LDL cholesterol = TC − HDL − (TRGs/5).

Insulin sensitivity

The fasting plasma glucose was divided by the fasting serum insulin to calculate the fasting glucose to insulin ratio (GIR) (17). The quantitative insulin sensitivity check index (QUICKI), the insulin area under the curve (I_{AUC120}), and the composite whole-body insulin sensitivity index [ISI(composite)] were also calculated (18–20). QUICKI was calculated as $1/(\log I_0 + \log G_0)$, and ISI(composite) was calculated according to the following formula: 10,000/square root of $[(G_0)\ (I_0)$ (mean serum insulin during OGTT) (mean plasma glucose during OGTT)]. I_{AUC120} was evaluated using the trapezoidal rule (20).

Evaluation of endothelial function by ultrasound

Endothelial function was evaluated between 0830 and 1000 h, with subjects fasted for at least 14 h. The subjects rested supine in a quiet, air-conditioned room (constant temperature, 22–25 C) for 30 min before endothelial function was assessed. Endothelial function was assessed in all women by measurement of flow-mediated dilation (FMD) of the brachial artery in response to hyperemia of the hand, a nitric oxide (NO)-mediated process (21).

FMD was measured as previously described (22), according to the method established by Celermajer *et al.* (23) and recently published guidelines (24). All studies were done by the same operator, who was unaware of the hormonal status of the women. Optimal imaging of the right brachial artery was obtained using an Echo-Doppler ultrasound (Ultrasound ATL, HDI 5000, Bophell, WA) and a 5–12-MHz transducer. Images were recorded on superVHS videotape (VCR Panasonic AG-MD 835, Osaka, Japan) for off-line analysis. Brachial artery diameter was measured by a blinded reader, at end-diastole coincident with R-wave on ECG, using electronic calipers from the anterior to the posterior m-line at a fixed distance from an anatomic marker.

Images were acquired at baseline (after 30-min supine rest), during hand hyperemia, *i.e.* 90 sec after deflation of a wrist cuff inflated to suprasystolic pressure (to at least 50 mm Hg above systolic pressure) for 5 min for measurement of FMD, and at 4 min after 400 μg sublingual glyceryl trinitrate (GTN) for measurement of endothelium-independent, nitrate-mediated dilation (NMD). All hemodynamic measurements were confirmed as having returned to baseline 15 min after release of the wrist cuff before administering GTN. Brachial artery blood flow was measured by continuous wave Doppler as the product of the Doppler time-velocity integral, heart rate, and brachial artery diameter measured at the time. FMD was calculated as the percent increase in arterial diameter during hyperemia compared with the diameter at rest, whereas hyperemic flow was measured as the peak flow at 15 sec after cuff release. Heart rate and blood pressure (by brachial sphygmomanometry) were also measured during the study.

Reproducibility: In our laboratory, the intra- and interobserver variability for repeated measurements of brachial artery diameter are 0.10 ± 0.11 and 0.09 ± 0.17 mm, respectively. In studies performed on two separate days (5–7 d apart) in eight subjects by a single operator, the within-subject coefficients of variation of the endothelium-dependent and endothelium-independent response were 4.9 and 3.2%, respectively.

Statistical analysis

Results are presented as mean ± SD. The clinical variables between PCOS and control women were compared using the unpaired Student's *t* test. To exclude potential interference of obesity, the comparison between PCOS and controls regarding hormonal, metabolic, and cardiovascular variables was performed by analysis of covariance (ANCOVA) taking BMI as a covariate. To compare differences among subgroups of PCOS women (lean, overweight, and obese), one-way ANOVA was used with the Scheffé test for *post hoc* comparisons. For not normally distributed variables, nonparametric tests were used; the Mann-Whitney *U* test for comparison between PCOS and controls and the Kruskal-Wallis test for comparison among PCOS subgroups. Partial correlation and multiple linear regression analysis were performed to assess the relationship between FMD and the studied clinical, metabolic, and hormonal characteristics. Subsequently, variables whose correlation with the FMD achieved near statistical significance ($P < 0.1$) were entered into a stepwise regression model to assess the magnitude of their individual effects on FMD. Not normally distributed variables underwent logarithmic transformation for the ANCOVA and the stepwise regression analysis. $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS 12.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Table 1 summarizes clinical and hormonal characteristics of the controls and the women with PCOS. PCOS women and controls had comparable age and BMI. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and FSH were similar in the two groups. PCOS women had significantly higher ($P < 0.05$) F-G score, LH, and total testosterone levels and significantly lower ($P < 0.005$) SHBG levels compared with controls. The LH to FSH ratio and the FAI were also significantly ($P < 0.005$) higher in PCOS than control women. Table 2 summarizes metabolic characteristics of the controls and the women with PCOS. PCOS women and controls had similar fasting glucose, insulin, and lipid levels. GIR, QUICKI, and ISI(composite) were slightly lower in PCOS than control women, but statistical significance was not reached ($P > 0.05$ for GIR and QUICKI and 0.054 for ISI). PCOS women had significantly higher ($P < 0.005$) I_{AUC120} than controls.

FMD was significantly lower in PCOS compared with control women (4.13 ± 2.72% *vs.* 9.09 ± 3.99%, $P < 0.0005$) (Table 3 and Fig. 1). The increase in hyperemic flow was not significantly different between the two groups. NMD was also lower in PCOS compared with control women (18.31 ± 5.86% *vs.* 24.47 ± 6.00%, $P < 0.0005$). Heart rate and blood pressure did not change during the study.

Partial correlation analysis between FMD and clinical, metabolic, and hormonal characteristics revealed that variables inversely associated with FMD were BMI (r = −0.272, $P = 0.03$), waist circumference (r = −0.292, $P = 0.02$), total testosterone (r = −0.279, $P = 0.03$), FAI (r = −0.256, $P = 0.05$), fasting insulin (r = −0.362, $P = 0.005$), I_{AUC120} (r = −0.382, $P = 0.004$), TC (r = −0.336, $P = 0.09$), LDL cholesterol (r = −0.313, $P = 0.02$), TRGs (r = −0.323, $P = 0.01$), and fasting glucose (r = −0.216, $P = 0.09$). Variables positively associated with FMD were fasting GIR (r = 0.289, $P = 0.03$) and the indices of insulin sensitivity QUICKI (r = 0.281, $P = 0.03$) and ISI(composite) (r = 0.432, $P = 0.001$). Stepwise regression analysis revealed that the I_{AUC120} accounted for 21% of the variance of FMD, whereas total testosterone and TC contributed an additional 10 and 9%, respectively ($P < 0.005$ for all).

Analysis of PCOS subgroups

Twenty-three women with PCOS were lean (BMI < 25 kg/m^2), 21 were overweight (BMI ≥ 25 and <30 kg/m^2), and 18 were obese (BMI ≥ 30 kg/m^2). As shown in Table 1, the lean and overweight were younger ($P < 0.05$) than the obese PCOS women. Obese had significantly higher ($P < 0.05$) SBP than lean PCOS women and significantly higher ($P < 0.05$) DBP than both lean and overweight PCOS women. No significant differences were found in F-G score, LH, FSH and testosterone levels, FAI, and LH to FSH ratio among these three subgroups. Lean women had significantly higher ($P < 0.05$) SHBG levels than both overweight and obese women.

As shown in Table 2, no significant differences among the three groups were found in fasting glucose and lipid levels, apart from TRGs, which were lower ($P < 0.05$) in lean compared with obese women. Fasting insulin was significantly lower ($P < 0.05$), and fasting GIR was significantly higher ($P < 0.05$) in lean compared with overweight and obese women and also in overweight compared with obese women. The indices QUICKI and ISI(composite) were significantly higher ($P < 0.05$), and I_{AUC120} was significantly lower ($P < 0.05$) in lean compared with overweight and obese PCOS women, whereas their difference between overweight and obese PCOS women did not reach statistical significance.

As shown in Table 3, a trend of deterioration in FMD from lean to overweight and obese PCOS women was observed (4.60 ± 2.63%, 4.28 ± 2.79%, and 3.35 ± 2.74%, respectively); however, no significant differences were found among the three subgroups. The increase in hyperemic flow and NMD did not differ among the subgroups.

In the group of lean PCOS women (n = 23), the only variable that had a borderline significant correlation with FMD was FAI (r = −0.395, $P = 0.06$). In the group of overweight PCOS (n = 21), variables that correlated with FMD

TABLE 1. Clinical and hormonal characteristics in women with PCOS and controls

	Control women (n = 17)	All PCOS women (n = 62)	Lean PCOS women (n = 23)	Overweight PCOS women (n = 21)	Obese PCOS women (n = 18)
Age (yr)	24.77 ± 5.66	22.69 ± 4.01	21.83 ± 3.66[b]	21.70 ± 3.70[c]	24.94 ± 4.08
Age range (yr)	18–35	18–35	18–32	18–32	18–35
Weight (kg)	67 ± 10	73 ± 14	59 ± 4[a,b]	73 ± 6[c]	89 ± 9
BMI (kg/m^2)	24.96 ± 4.26	27.59 ± 5.39	22.13 ± 1.75[a,b]	27.67 ± 1.42[c]	34.49 ± 2.69
Waist circumference (cm)	82.75 ± 10.18	84.50 ± 11.91	73.52 ± 4.46[a,b]	85.14 ± 7.96[c]	97.78 ± 7.63
WHR	0.81 ± 0.05	0.79 ± 0.06	0.76 ± 0.05[b]	0.79 ± 0.06	0.83 ± 0.06
SBP (mm Hg)	112 ± 6	116 ± 10	112 ± 8[b]	117 ± 7	121 ± 12
DBP (mm Hg)	70 ± 6	73 ± 7	69 ± 7[b]	72 ± 5[c]	78 ± 7
F-G score	2.50 ± 2.31	9.74 ± 3.46[e]	9.35 ± 2.44	10.19 ± 4.46	9.72 ± 3.38
LH (mIU/ml)	4.49 ± 2.39	8.32 ± 6.88[d]	8.87 ± 9.10	8.27 ± 6.73	7.67 ± 2.90
FSH (mIU/ml)	6.12 ± 1.71	5.50 ± 1.34	5.51 ± 1.29	5.39 ± 1.72	5.61 ± 0.94
LH/FSH ratio	0.75 ± 0.36	1.48 ± 1.15[e]	1.54 ± 1.51	1.51 ± 1.16	1.36 ± 0.43
Testosterone [ng/dl (nmol/liter)]	35.73 ± 9.80 (1.24 ± 0.34)	93.95 ± 33.72[e] (3.26 ± 1.17)	104.32 ± 35.16 (3.62 ± 1.22)	85.01 ± 38.33 (2.95 ± 1.33)	91.07 ± 22.48 (3.16 ± 0.78)
SHBG (nmol/liter)	51.80 ± 15.41	31.09 ± 12.95[e]	37.91 ± 10.50[a,b]	26.55 ± 15.46	27.16 ± 9.17
FAI (%)	2.57 ± 0.94	12.08 ± 7.47[e]	10.04 ± 3.73	13.81 ± 11.53	12.85 ± 5.04

WHR, Waist-to-hip ratio. Système International conversion factors: for testosterone (nmol/liter) 0.0347.
[a] $P < 0.05$ lean *vs.* overweight PCOS; [b] $P < 0.05$ lean *vs.* obese PCOS; [c] $P < 0.05$ overweight *vs.* obese PCOS; [d] $P < 0.05$ *vs.* controls; [e] $P < 0.005$ *vs.* controls.

TABLE 2. Metabolic characteristics in women with PCOS and controls

	Control women (n = 17)	All PCOS women (n = 62)	Lean PCOS women (n = 23)	Overweight PCOS women (n = 21)	Obese PCOS women (n = 18)
Fasting glucose [mg/dl (mmol/liter)]	90.09 ± 7.93 (5.00 ± 0.44)	89.01 ± 7.93 (4.94 ± 0.44)	86.49 ± 8.65 (4.80 ± 0.48)	89.73 ± 6.31 (4.98 ± 0.35)	90.99 ± 7.93 (5.05 ± 0.44)
Fasting insulin [μIU/ml (pmol/liter)]	10.01 ± 8.19 (69.53 ± 56.88)	11.21 ± 6.01 (77.88 ± 41.77)	6.94 ± 3.32[a,b] (48.20 ± 23.07)	11.20 ± 4.39[c] (77.75 ± 30.51)	16.46 ± 6.05 (114.28 ± 42.03)
Fasting GIR (mmol/pmol·10^{-2})	11.04 ± 7.32	8.56 ± 5.35	11.89 ± 5.31[a,b]	7.96 ± 5.35[c]	5.12 ± 5.28
QUICKI	0.42 ± 0.05	0.40 ± 0.05	0.44 ± 0.04[a,b]	0.39 ± 0.04	0.37 ± 0.03
I_{AUC120} [μIU/ml·min (pmol/liter·min)]	4,449 ± 3,142 (30,898 ± 21,821)	9,198 ± 6,623[d] (63,882 ± 45,994)	5,782 ± 3,391[a,b] (40,154 ± 23,552)	11,353 ± 8,793 (78,845 ± 61,067)	11,264 ± 5,617 (78,231 ± 39,009)
ISI (composite)	17.05 ± 8.89	12.31 ± 9.08	18.50 ± 10.70[a,b]	9.76 ± 5.74	7.41 ± 4.60
TC [mg/dl (mmol/liter)]	184.94 ± 41.70 (4.79 ± 1.08)	181.47 ± 32.05 (4.70 ± 0.83)	174.52 ± 30.12 (4.52 ± 0.78)	176.83 ± 30.89 (4.58 ± 0.80)	194.21 ± 33.98 (5.03 ± 0.88)
LDL [mg/dl (mmol/liter)]	118.53 ± 32.05 (3.07 ± 0.83)	116.60 ± 28.19 (3.02 ± 0.73)	108.88 ± 23.55 (2.82 ± 0.61)	114.29 ± 28.57 (2.96 ± 0.74)	128.57 ± 30.50 (3.33 ± 0.79)
HDL [mg/dl (mmol/liter)]	49.81 ± 13.13 (1.29 ± 0.34)	46.33 ± 8.49 (1.20 ± 0.22)	49.81 ± 8.11 (1.29 ± 0.21)	44.79 ± 8.11 (1.16 ± 0.21)	43.63 ± 8.11 (1.13 ± 0.21)
TRG [mg/dl (mmol/liter)]	82.30 ± 30.09 (0.93 ± 0.34)	92.04 ± 36.28 (1.04 ± 0.41)	80.53 ± 29.20[b] (0.91 ± 0.33)	90.27 ± 29.21 (1.02 ± 0.33)	109.73 ± 44.25 (1.24 ± 0.50)

Système International conversion factors: for glucose (mmol/liter) 0.0555; for insulin (pmol/liter) 6.945; for total, LDL and HDL cholesterol (mmol/liter) 0.0259; for TRG (mmol/liter) 0.0113.

[a] $P < 0.05$ lean *vs.* overweight PCOS; [b] $P < 0.05$ lean *vs.* obese PCOS; [c] $P < 0.05$ overweight *vs.* obese PCOS; [d] $P < 0.005$ *vs.* controls.

were total testosterone (r = −0.473, $P = 0.03$), LH to FSH ratio (r = −0.585, $P = 0.007$), fasting insulin (r = −0.498, $P = 0.03$), QUICKI (r = 0.511, $P = 0.025$), I_{AUC120} (r = −0.740, $P = 0.001$), and ISI(composite) (r = 0.679, $P = 0.004$). Stepwise regression analysis revealed that I_{AUC120} accounted for 58% of the variance of FMD, whereas testosterone contributed an additional 19% ($P < 0.01$ for both). In the group of obese PCOS (n = 18), variables that correlated with FMD were BMI (r = −0.537, $P = 0.021$), waist circumference (r = −0.554, $P = 0.017$), fasting insulin (r = −0.454, $P = 0.059$), TC (r = −0.554, $P = 0.017$), and LDL cholesterol (r = −0.543, $P = 0.002$). Stepwise regression analysis in this group revealed that TC and waist circumference accounted for 31 and 22% of the variance of FMD ($P < 0.05$ for both).

Discussion

PCOS is an intriguing reproductive disease, associated with hormonal adverse effects on the cardiovascular system. There is evidence suggesting that women with PCOS are at increased risk for cardiovascular disease, although prospective data regarding the risk of cardiovascular events in women with PCOS is scarce. A British epidemiologic study of 786 women with PCOS, who underwent ovarian wedge resection between 1930 and 1979, showed no increase in cardiovascular mortality when compared with expected age- and sex-specific mortality rates in the United Kingdom (5). A much larger study, however, showed an adjusted relative risk of 1.53 (95% confidence interval, 1.24–1.90) for coronary heart disease in women with irregular menstrual cycles, a marker of PCOS (25). A recent nested case-control study showed that lower SHBG and higher FAI (both features of PCOS) are found among postmenopausal women who develop cardiovascular events (26).

Several cross-sectional studies have also shown that PCOS is related with markers of increased cardiovascular risk. Christian *et al.* (10), using electron beam computer tomography, have recently demonstrated coronary artery calcium, a marker of coronary atherosclerosis, to be more prevalent in women with PCOS (aged 30–45 yr) than in obese or nonobese controls. Furthermore, young women with PCOS (aged 24 ± 6 yr) were found to have an increased left ventricular mass index and diastolic dysfunction (27). In a study of 143 women undergoing cardiac catheterization, ultrasound polycystic ovary appearance was detected in 42% of those; these patients more frequently exhibited extensive coronary artery disease (8). Other markers of increased cardiovascular risk, such as homocysteine (28) and C-reactive protein (27, 29), were also elevated in women with PCOS. Finally, using

TABLE 3. Vascular data in women with PCOS and controls

	Control women (n = 17)	All PCOS women (n = 62)	Lean PCOS women (n = 23)	Overweight PCOS women (n = 21)	Obese PCOS women (n = 18)
Baseline EDD (mm)	3.09 ± 0.47	3.12 ± 0.39	2.95 ± 0.34	3.23 ± 0.34	3.20 ± 0.43
Absolute Δ in EDD with hyperemia (mm)	0.28 ± 0.14	0.13 ± 0.08[a]	0.13 ± 0.08	0.13 ± 0.09	0.11 ± 0.08
Δ in EDD with hyperemia—FMD [% (range)]	9.09 ± 3.99 (1.35–16.10)	4.13 ± 2.72[a] (−2.10–10.80)	4.60 ± 2.63 (0.0–10.80)	4.28 ± 2.79 (−0.75–8.40)	3.35 ± 2.74 (−2.10–8.62)
Absolute Δ in EDD with GTN (mm)	0.73 ± 0.16	0.57 ± 0.17[a]	0.57 ± 0.20	0.57 ± 0.15	0.57 ± 0.14
Δ in EDD with GTN—NMD [% (range)]	24.47 ± 6.00 (17.74–38.10)	18.32 ± 5.86[a] (7.14–31.13)	19.16 ± 6.73 (7.14–28.57)	17.73 ± 5.57 (8.75–31.13)	17.95 ± 5.16 (9.47–29.55)
Baseline flow (ml/min)	30 ± 11	30 ± 24	22 ± 13	39 ± 34	31 ± 16
Peak hyperemic flow (ml/min)	169 ± 91	148 ± 72	133 ± 56	175 ± 96	137 ± 49

EDD, End-diastolic diameter; Δ, change.

[a] $P < 0.005$ *vs.* controls.

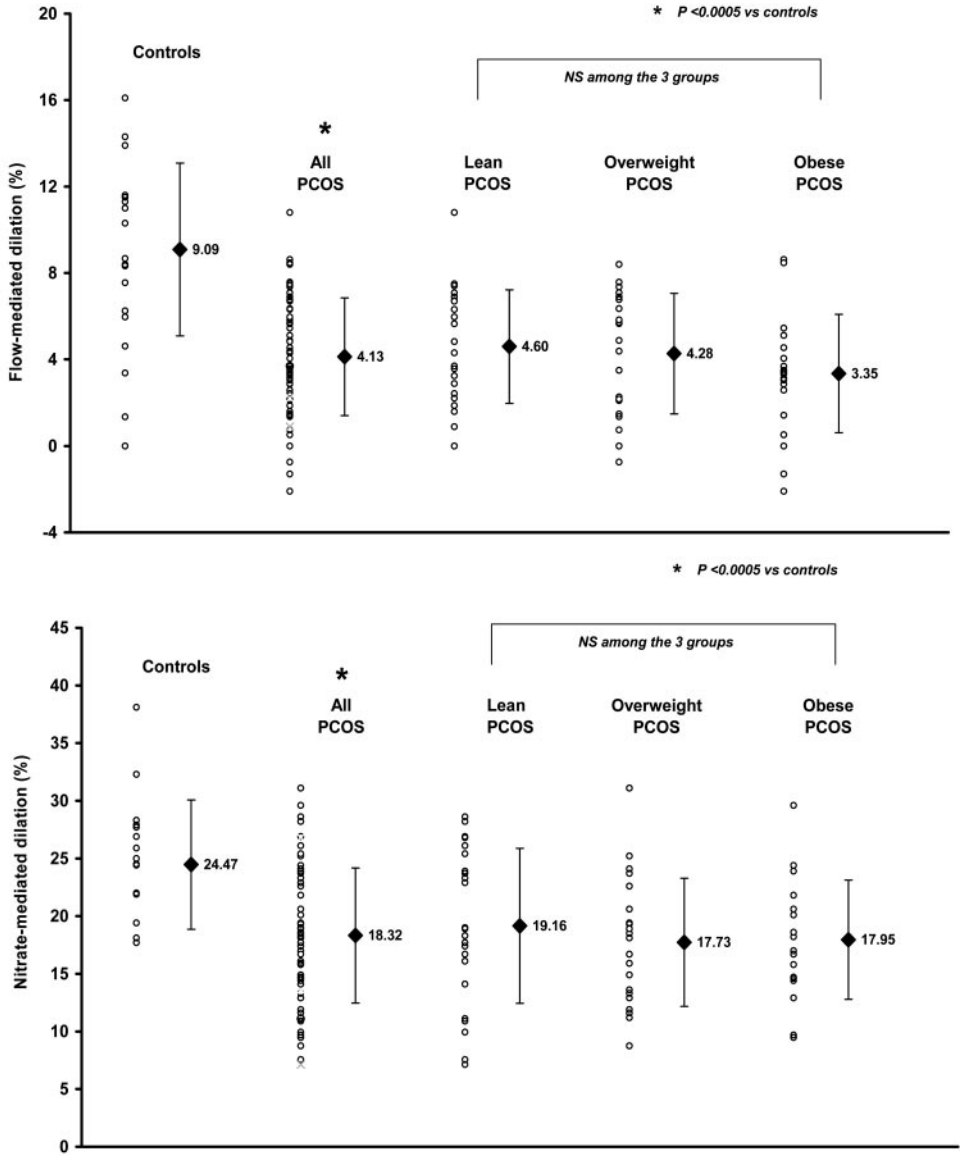

FIG. 1. Vascular function in control women, all women with PCOS, and lean, overweight, and obese women with PCOS. *Upper panel*, FMD (percentage change in brachial artery diameter during hand hyperemia; endothelium-dependent response). *Lower panel*, NMD (percentage change in brachial artery diameter after GTN; endothelium-independent response).

a risk model analysis, Dahlgren *et al.* (30) suggested that women with PCOS have a 4- to 7-fold higher risk of myocardial infarction compared with age-matched controls.

Assessment of vascular endothelial function may serve as an integrating index of cardiovascular risk factor burden (24). Endothelium regulates vascular tone through the release of vasodilators, such as NO, and vasoconstrictors, such as endothelin, in response to physical and chemical stimuli. Increased blood flow is an important stimulus for endothelium-mediated vasodilation (FMD). FMD can be assessed noninvasively using high-frequency ultrasound to measure changes in brachial artery diameter in response to hyperemic flow, induced by a 5-min pressure cuff arterial occlusion at the wrist; these changes during hand hyperemia are mainly due to endothelial release of NO (21). Brachial artery FMD correlates with the assessment of endothelial function in the coronary circulation (31), which has been shown to represent an independent predictor of cardiovascular events (32). Endothelial dysfunction, demonstrated as reduced FMD, has been associated with the presence of cardiovascular risk factors and contributes to the development of atherosclerosis (24).

To investigate whether young women with PCOS have endothelial dysfunction, we assessed FMD in these women and in healthy controls, matched as a group for age and BMI. Obesity, a known cardiovascular risk factor, is frequently encountered in women with PCOS (2), and its presence may play an important role in the endocrine, metabolic, and cardiovascular characteristics of these women (20). In the present study, the control group did not include many obese subjects. Therefore, to exclude potential interference of obesity, the hormonal, metabolic, and cardiovascular characteristics in the two groups (controls *vs.* PCOS) were compared taking BMI as a covariate. Our findings demonstrated that women with PCOS have significant endothelial dysfunction at an early age, *i.e.* in their early 20s. FMD was significantly

lower (reduced by ~50%) in young PCOS women (aged 23 ± 4 yr) compared with regularly menstruating controls.

The relationship of BMI with endothelial function in PCOS was further assessed by stratifying women with PCOS according to BMI into lean, overweight, and obese subgroups. Although a trend of deterioration in endothelial function with increasing BMI was observed (FMD, 4.60 ± 2.63%, 4.28 ± 2.79%, and 3.35 ± 2.74% in lean, overweight, and obese women with PCOS, respectively), these differences among groups were not significant, suggesting that the process of endothelial dysfunction in PCOS is largely independent of obesity. This was also suggested by the finding that, although BMI and waist circumference were inversely related to FMD in correlation analysis ($r = -0.272$, $P = 0.032$ and $r = -0.292$, $P = 0.021$, respectively), these parameters were not among the independent predictors of FMD in stepwise regression analysis. Of note, all PCOS women and controls in our study were nonsmokers and were not receiving any vitamins, antioxidants, or cardiovascular medications, factors that may affect FMD.

Previously reported data on endothelial dysfunction in PCOS women have been conflicting. Consistent with our results, Paradisi *et al.* (12), using invasive methodology, demonstrated endothelial dysfunction (50% reduction in leg blood flow responses to intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride) and resistance to the vasodilating action of insulin in obese women with PCOS compared with age- and BMI-matched healthy controls. Using noninvasive methodology (FMD), we demonstrated a similar degree of endothelial dysfunction in a group of PCOS women with apparently more favorable characteristics such as younger age and lower BMI. In contrast to our findings, Mather *et al.* (11), using FMD, reported no evidence of impairment of endothelium-dependent function in obese PCOS compared with age-matched, nonobese healthy women despite marked differences in BMI, insulin resistance, hyperandrogenism, blood pressure, and TC and LDL cholesterol. There are several differences between the two studies; the present study included a larger population of PCOS women with different baseline characteristics. In agreement with our findings, two studies have recently published data on endothelial function in young PCOS women. Orio *et al.* (13) have demonstrated impaired FMD (by approximately 20%) in young (mean age, 22 yr), lean women with PCOS compared with age- and BMI-matched controls, although this difference between groups may be partly due to the greater (by approximately 10%) baseline brachial artery diameter in women with PCOS. Tarkun *et al.* (14) have also reported impairment of FMD and NMD (by approximately 32 and 21%, respectively) in young (mean age, 23.5 yr), nonobese women with PCOS compared with age- and BMI-matched controls. In both these latter studies, brachial rather than wrist occlusion has been used for the measurement of FMD, a process not entirely mediated by NO (21); this may account for the difference in the degree of endothelial dysfunction reported in these studies compared with the present one.

Apart from obesity, insulin resistance, hyperandrogenism, dyslipidemia, and hypertension are all features of the PCOS that may affect endothelial function. In addition, these metabolic and endocrine parameters are also influenced by obesity; thus, metabolic and hormonal characteristics were compared in the two groups using ANCOVA, taking BMI as a covariate. In our study, PCOS women demonstrated a greater insulin response to an oral glucose load (I_{AUC120}) compared with controls, suggesting insulin resistance. PCOS women also had a higher F-G score, higher levels of total testosterone, FAI, LH, and LH to FSH ratio, and lower levels of SHBG, thus reflecting hyperandrogenism. Women with PCOS had no significant differences in resting blood pressure or fasting lipids compared with controls, in keeping with other reports (33, 34).

In an effort to clarify the determinants of FMD in these PCOS women, clinical, hormonal and metabolic variables whose correlation with FMD achieved near statistical significance ($P < 0.1$) were entered in a stepwise regression analysis model. An index of insulin resistance (I_{AUC120}), total testosterone, and TC were predictive of endothelial function, accounting for 21, 10, and 9%, respectively, of the variance in FMD. Insulin resistance has been previously linked to endothelial dysfunction in nonobese women with PCOS (13, 14). Endothelial dysfunction has also been associated with increased androgen levels and insulin resistance in obese PCOS women (12).

The present study also investigated metabolic and hormonal characteristics in lean, overweight, and obese PCOS women to assess their effect on endothelial function. Obese PCOS were older than both lean and overweight women and had higher TRG levels compared with the lean. The insulin response to an oral glucose load (I_{AUC120}) was significantly higher, whereas the indices of insulin sensitivity, QUICKI, and ISI(composite), were significantly lower in overweight and obese PCOS compared with lean ones, in accordance with previously reported data (19). SHBG levels were significantly higher in lean compared with overweight and obese women, whereas total testosterone and FAI did not differ among the three subgroups. Using partial correlation and stepwise regression analysis, determinants of endothelial function were identified in each of the three subgroups. In lean PCOS women, FMD was not significantly related to any clinical or metabolic variables, possibly apart from hyperandrogenism; FAI was the only variable that demonstrated an almost significant inverse correlation with endothelial function ($P = 0.06$). An inverse correlation between FAI and carotid intima-media thickness has been previously reported in young, lean PCOS women (13). In overweight PCOS, insulin resistance was strongly related to endothelial dysfunction, whereas hyperandrogenism also played an important role, in accordance with previous reports (12). I_{AUC120} and total testosterone could explain 58 and 19%, respectively, of the FMD variance in the overweight group. Finally, in obese women, dyslipidemia and central adiposity were important determinants of endothelial dysfunction; TC and waist circumference accounted for 31 and 22%, respectively, of the variance in FMD.

NMD was also modestly impaired in PCOS compared with control women (18.32 ± 5.86% *vs.* 24.47 ± 6.00%, $P < 0.0005$, reduced by about 25%), in agreement with a recently published study (14). Similar findings were previously reported in children with diabetes or familial hypercholester-

olemia (35, 36) and adult patients with heart failure (37) and coronary heart disease (38). The vasodilatory response to GTN is considered to be a function of both smooth muscle relaxation (direct action of GTN to the smooth muscle) and the response to hyperemia caused by GTN-induced dilation of the resistance vessels (35). Loss of this hyperemic flow component to the GTN response may explain the mild impairment of NMD seen in the PCOS group; however, a smooth muscle abnormality in the conduit arteries of these women cannot be excluded. In agreement with this, others have previously reported vascular abnormalities in PCOS. Women with PCOS, in comparison with control women, demonstrate greater carotid intima-media thickness (9, 13) and increased stiffness in the carotid (39) and brachial (40) arteries, whereas a paradoxical constrictor response to GTN in the uterine artery has also been reported in women with PCOS (41).

In conclusion, asymptomatic women with PCOS have impaired endothelial function at an early age (*i.e.* early 20s), suggesting an increased risk for early onset cardiovascular disease. Endothelial dysfunction in PCOS does not depend on obesity, and its determinants vary among lean, overweight, and obese women. Women with PCOS may gain particular benefit from early screening for cardiovascular risk factors and cardioprotective measures directed toward improving endothelial function, including diet, physical exercise, and possibly insulin sensitizers or androgen-lowering agents.

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