Polycystic Ovary Syndrome Is Associated With Endothelial Dysfunction

Giancarlo Paradisi, MD; Helmut O. Steinberg, MD; Annette Hempfling, RN; Jessica Cronin, RN; Ginger Hook, RN; Marguerite K. Shepard, MD; Alain D. Baron, MD

Background—We recently reported endothelial dysfunction as a novel cardiovascular risk factor associated with insulin resistance/obesity. Here, we tested whether hyperandrogenic insulin-resistant women with polycystic ovary syndrome (PCOS) who are at increased risk of macrovascular disease display impaired endothelium-dependent vasodilation and whether endothelial function in PCOS is associated with particular metabolic and/or hormonal characteristics.

Methods and Results—We studied leg blood flow (LBF) responses to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride (MCh) and to euglycemic hyperinsulinemia in 12 obese women with PCOS and in 13 healthy age- and weight-matched control subjects (OBW). LBF increments in response to MCh were 50% lower in the PCOS group than in the OBW group ($P < 0.01$). Euglycemic hyperinsulinemia increased LBF above baseline by 30% in the PCOS and 60% in OBW group ($P < 0.05$ between groups). Across all subjects, the maximal LBF response to MCh exhibited a strong inverse correlation with free testosterone levels ($r = -0.52$, $P < 0.007$). This relationship was stronger than with any other parameter, including insulin sensitivity.

Conclusions—PCOS is characterized by (1) endothelial dysfunction and (2) resistance to the vasodilating action of insulin. This endothelial dysfunction appears to be associated with both elevated androgen levels and insulin resistance. Given the central vasoprotective role of endothelium, these findings could explain, at least in part, the increased risk for macrovascular disease in women with PCOS. (Circulation. 2001;103:1410-1415.)

Key Words: blood flow ■ insulin ■ glucose ■ nitric oxide ■ hormones

We recently reported that obese insulin-resistant subjects, as a group, are characterized by impaired insulin-mediated vasodilation, reduced vasodilation to the endothelium-dependent vasodilator methacholine chloride (MCh), and blunting of the effect of insulin to enhance endothelium-dependent vasodilation. The contribution of endothelial dysfunction to the increased risk of atherosclerosis and cardiovascular disease in insulin-resistant subjects has been discussed extensively. Interestingly, on closer analysis of our data, we observed that obese women (OBW), in contrast to obese men, display endothelial function (MCh-induced vasodilation) that is similar to that of lean men. These sex differences suggested that sex hormones could modulate endothelial function. This hypothesis is consistent with epidemiological and experimental reports describing a protective effect of female hormonal patterns on the macrovasculature. Cardiovascular events are less prevalent in premenopausal women and in women receiving estrogen replacement than in postmenopausal women or men. In particular, studies have shown that estrogen replacement therapy improves endothelium-dependent vasodilation, with favorable influences on insulin sensitivity and lipid profile.

In contrast, androgens are generally considered to decrease glucose tolerance, induce insulin resistance, and increase cardiovascular risk in women as well as in men.

Women with polycystic ovary syndrome (PCOS) represent an intriguing biological experiment of nature that illustrates hormonal effects on cardiovascular risk. These patients, characterized by elevated testosterone levels, hirsutism, and oligomenorrhea, are typically obese and insulin resistant and are suspected to be at increased risk for cardiovascular disease. Therefore, this premenopausal subject group presents an interesting and propitious constellation of clinical findings that allows one to evaluate whether a male hormonal pattern abrogates the protective sex effects on endothelial function in OBW.

Thus, the present study was designed to test the hypothesis that women with PCOS display endothelial dysfunction and if so, to determine whether this abnormality is related to elevated testosterone levels.

Methods

Subjects

The characteristics of the study groups are shown in Table 1. Twelve OBW with PCOS were recruited for the study. PCOS was diagnosed...
TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>OBW (n=13)</th>
<th>PCOS (n=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.0±2.2</td>
<td>29.1±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.8±1.7</td>
<td>36.7±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.84±0.01</td>
<td>0.89±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>47.1±1.8</td>
<td>46.4±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Basal glucose, mg/dL</td>
<td>89.4±2.3</td>
<td>91.6±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Basal insulin, µU/mL</td>
<td>14.4±1.2</td>
<td>26.5±4.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>179±8</td>
<td>182.8±9</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>42.6±1.6</td>
<td>33.9±1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>120±8</td>
<td>123±9</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>85±8</td>
<td>144±21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Free fatty acids, µmol*</td>
<td>563±65</td>
<td>566±88</td>
<td>NS</td>
</tr>
<tr>
<td>FT, pg/mL</td>
<td>1.08±0.17</td>
<td>4.17±0.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total testosterone, ng/dL</td>
<td>36.8±5.9</td>
<td>90.1±10.6</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>96.7±23.5</td>
<td>57.2±6.8</td>
<td>NS</td>
</tr>
<tr>
<td>DHEAS, µmol/L</td>
<td>2.77±0.77</td>
<td>2.06±0.29</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>94.9±2.7</td>
<td>99.5±3.3</td>
<td>NS</td>
</tr>
</tbody>
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DHEAS indicates dehydroepiandrosterone sulfate. Values are mean±SEM.

*Regions of significant differences. Bold values indicate differences between study groups.

by an elevation of free testosterone (FT) levels, associated with hirsutism and amenorrhea or chronic oligomenorrhea (<6 periods per year). Nonovarian causes of hyperandrogenism were not formally excluded. Obesity was defined as body mass index (BMI)=30, and fat content was determined by underwater weighing. The weight limit for accurate determination of body composition with our absorptiometry (DXA; system software 1.2; Lunar DPX-L). The 30, and fat content was determined by dual-energy x-ray absorptiometry (DXA; system software 1.2; Lunar DPX-L). The 30, and fat content was determined by dual-energy x-ray absorptiometry (DXA; system software 1.2; Lunar DPX-L). The 30, and fat content was determined by dual-energy x-ray absorptiometry (DXA; system software 1.2; Lunar DPX-L). The 30, and fat content was determined by dual-energy x-ray absorptiometry (DXA; system software 1.2; Lunar DPX-L). The 30, and fat content was determined by dual-energy x-ray absorptiometry (DXA; system software 1.2; Lunar DPX-L). The

Hemodynamic Measurements
LBF, MAP, and heart rate measurements were obtained at baseline and during intrafemoral artery infusion of MCh at sequential doses of 5, 10, and 15 µg/min. All hemodynamic measurements were repeated after ~200 minutes of euglycemic hyperinsulinemia.

Whole-Body Glucose Disposal: Leg Glucose Uptake
Whole-body glucose disposal was assessed with the euglycemic hyperinsulinemic clamp technique as previously described. Each clamp was performed during a 4-hour square-wave infusion of insulin at a rate of 120 mU·m⁻²·min⁻¹.

Leg glucose uptake (LGU) was calculated as the product of the blood arteriovenous glucose difference (AVGΔ) and LBF, LGU=AVGΔ×LBF. Plasma glucose was converted to blood glucose by the following formula: blood glucose=plasma glucose×(1−0.3×hematocrit).

Statistical Analysis
Results are shown as the mean±SEM. Leg vascular resistance (LVR) was defined as LVR=MAP/LBF and is expressed in arbitrary units (U). Changes in blood flow are expressed as percent change (%Δ) to adjust for the differences at baseline. GDR is expressed as mg·kg lean body mass⁻¹·min⁻¹. Two-way ANOVA was used to compare the responses to the graded MCh infusions. One-tailed paired and unpaired t tests were performed as appropriate for comparisons within or between groups. Simple linear regression and partial correlation analysis were performed to assess the relationship between the maximal increase in LBF in response to the intrafemoral artery infusions of MCh and the hormonal and metabolic characteristics studied. Subsequently, variables whose correlation with the maximum LBF response to the intrafemoral artery infusions of MCh achieved near statistical significance (P<0.1) were entered into a stepwise regression model to assess the magnitude of their individual effects on the maximum LBF response to the intrafemoral artery infusions of MCh. Statistical significance was accepted at a level of P<0.05.

Results
Rates of Whole Body Glucose Uptake and LGU
As expected, glucose disposal rate (GDR) was lower in the PCOS subjects than in OBW (4.78±0.48 mg·kg⁻¹·min⁻¹, P<0.0006, respectively), despite the higher insulin levels (358±22 versus 294±19 µU/mL, P<0.05). This difference in insulin-stimulated glucose metabolism between groups was even more striking when GDR was normalized to lean mass (P<0.0005, Figure 1A).

To further evaluate the ability of insulin to stimulate LBF and muscle glucose uptake, we analyzed LGU in both groups studied. As illustrated (Figure 1B), under basal conditions LGU was similar in OBW and PCOS women (P=NS) as a result of similar LBF and AVGΔ (0.28±0.02 versus 0.31±0.04 L/min, P=NS, and 2.1±0.3 versus 1.7±0.3 mg/dL, P=NS, respectively). Conversely, during steady-state euglycemic hyperinsulinemia, LGU in PCOS was nearly 40% lower than in OBW (P<0.01). The differences in LGU between the 2 groups reflect their differences in LBF and AVGΔ, for which PCOS displayed both smaller increments in muscle glucose extraction, as indicated by their lower AVGΔ (21.4±1.8 versus 14.9±2.4 mg/dL, P<0.03 versus OBW), and somewhat reduced rates of insulin-mediated LBF (0.43±0.04 versus 0.37±0.03 L/min, P=0.10 versus OBW).

Protocol
At ~7:00 AM, after an overnight 14-hour fast, a catheter was inserted into the antecubital vein for infusions of substances. Subsequently, the right femoral artery and vein were cannulated. A 6F sheath (Cordis Corp) was placed in the right femoral vein to allow the insertion of a custom-designed 5F double-lumen thermodilution catheter (Baxter Scientific, Edwards Division) to measure leg blood flow (LBF) as previously described. The right femoral artery was cannulated with a 5.5F double-lumen catheter (Arrow International) to allow simultaneous infusion of substances through the proximal (most caudad) and invasive blood pressure monitoring through the distal (most cephalad) port. Heart rate and mean arterial blood pressure (MAP) were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor (VSM 1, Physiocontrol).
Lipids
Lipid patterns of PCOS and OBW are shown in Table 1. PCOS had lower HDL-cholesterol levels and nearly 40% higher triglyceride levels with respect to OBW (P,0.01). No differences were seen between the 2 groups with regard to the other lipids.

Hemodynamic Data
MAP was somewhat higher in PCOS, but this difference did not reach statistical significance (Table 1). MAP did not change in response to euglycemic hyperinsulinemia in the PCOS group. In OBW, conversely, MAP displayed an 7% decrease from basal values (P,0.003). In response to the intrafemoral artery infusion of MCh, MAP was unchanged under either basal or hyperinsulinemic conditions in both groups.

Vascular Reactivity
Basal LBF was 0.28±0.02 and 0.31±0.04 L/min in OBW and PCOS, respectively (P=NS). Euglycemic hyperinsulinemia induced a 58.6±15.5% increase in LBF in insulin-sensitive OBW (P<0.003), whereas in insulin-resistant PCOS, the increment in LBF was only 29.5±14.8% (P=NS). LBF showed a significant dose-dependent increase (P<0.01) in response to the intrafemoral artery infusion of MCh in both groups. Compared with OBW, LBF increments in response to the intrafemoral artery infusion of MCh were on average 50% lower (Figure 2A) in the PCOS group (P<0.01). The diminished response to the intrafemoral artery infusion of MCh suggests that endothelium-dependent vasodilation is impaired in women with PCOS. During steady-state euglycemic hyperinsulinemia, the LBF rise above baseline was 50% lower in PCOS than in OBW (P<0.05) (Figure 3A). Thus, the blunted effect of insulin to induce vasodilation in PCOS suggests that these women display resistance to the vascular action of insulin.

Basal LVR was similar in the OBW and PCOS groups (354±23 versus 354±31 U, respectively, P=NS). Con-

Figure 1. A, Rates of whole-body insulin-stimulated glucose disposal in control (open boxes) and PCOS (solid circles) subjects during 240 minutes of euglycemic hyperinsulinemia (120 mU·m²·min⁻¹). B, Rates of LGU in control (open boxes) and PCOS (solid circles) subjects under basal conditions and after 200 minutes during steady-state euglycemic hyperinsulinemia.

Figure 2. LBF increments (A) and LVR decrements (B) relative to baseline (%Δ) in response to graded intrafemoral artery infusions of MCh in control (open boxes) and PCOS (solid circles) subjects.

Figure 3. Percent increase above baseline in LBF (A) and percent reduction below baseline in LVR (B) in response to euglycemic hyperinsulinemia (120 mU·m²·min⁻¹) in control (open boxes) and PCOS (solid circles) subjects.
versely, during euglycemic hyperinsulinemia, LVR was significantly lower in OBW than in PCOS (224±18 versus 279±27 U, P<0.05). The percent decrease in LVR below baseline in response to euglycemic hyperinsulinemia was also more marked in OBW than in PCOS (P<0.03, Figure 3B). In response to the intrafemoral artery infusions of MCh, LVR decreased in a dose-dependent manner in both groups, and changes in LVR mirrored the changes in LBF. Changes in LVR were significantly more pronounced in OBW than in PCOS women (P<0.01, Figure 2B).

Correlational Analyses

The results of the regression analysis (Table 2) suggest that levels of FT and total testosterone are directly associated with the magnitude of impairment of endothelium-dependent vasodilation. A strong negative association was also found between the degree of obesity (as gauged by BMI) and the maximum LBF response to MCh. GDR (insulin sensitivity) and the maximum LBF response to MCh were directly associated; however, this relation did not quite achieve statistical significance (P=0.062). Because FT, BMI, and GDR were interrelated, we performed stepwise regression analysis to evaluate their independent contribution to predict the endothelium-dependent vasodilation. Stepwise regression analysis (Table 3) revealed that FT accounted for 27% of the variance of the maximum LBF response to MCh under basal conditions (Figure 4), whereas BMI contributed an additional 18%. GDR did not contribute to the regression model.

Endothelium-dependent vasodilation was examined after an overnight fast (basal insulin levels) by measuring the LBF response to graded intrafemoral artery infusions of MCh. MCh-induced vasodilation is a well-established method to assess endothelial relaxing function and is considered to largely reflect NO production/release.18 Therefore, the data suggest that diminished vasodilatory response to MCh in women with PCOS is due, at least in part, to impaired production/release of NO.

In keeping with other reports, women with PCOS exhibited a remarkable defect in insulin-stimulated glucose uptake, with ≈38% lower whole-body GDRs.19 As stated in the Methods section, control subjects were not studied at a consistent phase of the menstrual cycle, whereas women with PCOS were studied mostly in anovulatory status. If endothelial function and insulin sensitivity vary during the menstrual cycle,20,21 this could confound our results. When we analyzed the data from OBW according to the phase of the menstrual cycle, however, we found that endothelial function, as expressed by the maximal LBF response to MCh and steady-state GDRs of 7 women who were in the follicular phase, was similar to that of the 6 women studied during the luteal phase (232±41% versus 234±44%, P=NS, and 15.1±1 versus

| TABLE 2. Correlational Analyses between the Maximum Percent Increase in LBF in Response to Graded Intrafemoral Artery Infusions of MCL and Various Relevant Parameters |
|-------------------------------|-----------------|-----------------|
| Independent variables | Basal (n=25) |
| ----------------- | r | P |
| BMI, kg/m² | −0.46 | <0.02 |
| Waist/hip ratio | −0.35 | 0.087 |
| FT, pg/mL | −0.52 | <0.007 |
| Total testosterone, ng/dL | −0.50 | <0.01 |
| Estradiol, pg/mL | −0.19 | 0.34 |
| GDR, mg·kg lean mass⁻¹·min⁻¹ | 0.38 | 0.062 |
| Basal insulin, μU/mL | −0.24 | 0.25 |
| Cholesterol, mg/dL | −0.16 | 0.44 |
| HDL cholesterol, mg/dL | 0.33 | 0.104 |
| LDL cholesterol, mg/dL | −0.13 | 0.54 |
| Triglyceride, mg/dL | −0.27 | 0.18 |
| Free fatty acids, μmol* | 0.17 | 0.51 |
| MAP, mm Hg | −0.24 | 0.24 |

*n=16.

| TABLE 3. Stepwise Regression Analysis for the Relationship between FT and BMI and the Maximum Change in LBF in Response to the Endothelium-Dependent Vasodilator MCH |
|-------------------------------|-----------------|-----------------|
| Independent variables | Regression Coefficients | R² | P |
| FT | −30.24±9.69 | 0.27 | 0.005 |
| BMI | −8.26±3.05 | 0.45 | 0.013 |
| Intercept | 554±112 | <0.0001 |

Figure 4. Linear regression analysis between the maximum LBF response (% Δ) to intrafemoral artery infusions of MCh and FT levels across all subjects (solid circles, OBW; open circles, PCOS).
15.3±1.5 mg · kg lean body mass⁻¹ · min⁻¹, P=NS, respectively). Therefore, it is not likely that the differences observed in endothelial function and insulin sensitivity between OBW and PCOS were greatly influenced by differences in menstrual cycle phase.

It is interesting to note that GDRs were directly related to the magnitude of the action of insulin to vasodilate (r=0.421, P<0.04). These data are consistent with our previously published reports in non-PCOS subjects supporting a strong association (metabolic coupling) between the vascular action of insulin and its overall effect on glucose metabolism. Reduced insulin-mediated glucose disposal exhibited by PCOS was reflected at the level of skeletal muscle. Indeed, whereas LGU was similar in the 2 groups under basal conditions, during hyperinsulinemia, LGU in PCOS was 40% lower than in OBW. This reduction of insulin-stimulated skeletal muscle glucose uptake was mostly due to reductions in glucose extraction (reflected by reduced AVGΔ) and to a lesser degree to reduced rates of glucose and/or insulin delivery (reflected by lower rates of LBF).

The previously reported relationship between endothelial dysfunction and insulin resistance, it follows logically that the marked insulin resistance displayed by women with PCOS could account in large part for the endothelial dysfunction. It is important to consider, however, that insulin resistance is not the only characteristic of PCOS, which may have a detrimental effect on endothelial function. Androgens, dyslipidemia, obesity, and hypertension all may affect endothelial function. Androgens, dyslipidemia, obesity, and hypertension all may affect endothelial function. Therefore, we analyzed all the variables to assess which of these play a major determinant role in the endothelial dysfunction displayed by women with PCOS.

As in our previous study, we observed a strong negative correlation between BMI and the peak vasodilation in response to MCh. BMI, together with FT, was shown to be a powerful predictor of endothelial function, being able to explain 18% and 27%, respectively, of the variance in maximal endothelium-dependent vasodilation. Thus, obesity appears to have a significant modulating effect on endothelial function in women, independent of their hormonal status. In addition, given a certain degree of adiposity, hyperandrogenism appears to have an additive negative effect on endothelial function.

By design, women with PCOS exhibited total testosterone and FT levels 2- and 4-fold higher, respectively, than those observed in OBW women. Perhaps the most intriguing finding in our study is the strong negative correlations between both total testosterone and FT and the peak vasodilation induced by MCh across all subjects, suggesting that increased androgen levels may impair endothelial function.

As stated above, elevated FT levels were part of the selection criteria for PCOS. Therefore, to exclude the possibility that the correlation between FT and peak vasodilation induced by MCh could be influenced by other factors associated with PCOS, we performed a further analysis adjusting the relation for the variables that differed significantly between groups (eg, GDR, fasting insulin, HDL, triglyceride). This analysis revealed that the correlation remained significant (r=−0.41, P<0.05). We did not measure serum sex hormone–binding globulin levels. Given that insulin resistance leads to a decrease in serum sex hormone–binding globulin, resulting in elevated FT levels, this could possibly strengthen the negative relation between FT and endothelial function.

Consistent with our results, Herman et al reported that androgen deprivation in men is associated with enhanced endothelium-dependent vasodilatation. Conversely, it has also been shown that estrogen supplementation failed to improve endothelium-dependent vasodilation in men but did so in women. Therefore, it seems plausible that a deleterious effect of androgens rather than simply beneficial effects of estrogens may contribute to the observed sex difference in the risk of macrovascular disease in healthy subjects. In women with PCOS, androgen levels appear to be major contributors to endothelial dysfunction and, as such, a major potential mechanism of macrovascular disease in these patients.

In keeping with other reports, total and LDL cholesterol levels in PCOS subjects were similar to those observed in weight-matched control women. Moreover, there was no relationship between these lipids and FT or with steady-state GDRs (insulin sensitivity). Therefore, it is highly unlikely that these lipid parameters could account for the differences in endothelial function observed between our study groups. Women with PCOS had lower HDL cholesterol and higher triglyceride levels than control women. As in our previous reports, these lipids did not correlate with the peak endothelium-dependent vasodilation induced by MCh, although one cannot completely exclude an indirect contribution. Free fatty acid levels were similar in control and PCOS subjects and thus also cannot account for the difference in endothelium-dependent vasodilation observed between groups. In keeping with most studies, we found that resting blood pressure was not significantly higher in women with PCOS than in weight- and age-matched control subjects. Moreover, blood pressure did not correlate with the maximum response to intrafemoral artery infusions of MCh. Thus, it is not likely that the large difference in endothelial function observed between the study groups is related to the modest differences in blood pressure levels.

In conclusion, this study provides the first evidence that PCOS is characterized by endothelial dysfunction. This endothelial dysfunction appears to be most strongly associated with both elevated androgen levels and obesity/insulin resistance. Given the central vasoprotective role of the endothelium, these findings could explain, at least in part, the suspected increased risk for macrovascular disease in women with PCOS. Although the mechanism by which male hormone pattern/insulin resistance determines endothelial dysfunction remains to be defined, the data support the hypothesis that clinical strategies aimed at reducing both androgens and insulin resistance would have the most beneficial cardioprotective effects in women with PCOS.

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